CALCIUM and PHOSPHATE METABOLISM

in

CATTLE and SHEEP

by

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A thesis submitted for the degree of

Doctor of Veterinary Medicine and Surgery

of the

University of Edinburgh.

March, 1964.

CONTENTS

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The intensive investigation of calcium and phosphate metabolism in ruminants dates from 1925 when Dryerre and Greig postulated that hypocalcaemia might be associated with the condition of milk fever in cattle. This condition occurs in older cows about the time of calving and can be successfully treated in most cases by calcium replacement therapy. Experimental support for Dryerre and Greig's postulate was soon forthcoming (Little and Wright, 1925; Dryerre and Greig, 1928) and investigations were no doubt facilitated by advances in biochemical techniques.

Although it had been possible to estimate the calcium and phosphate in serum for a number of years (Pribram, 1871; Rona and Takahasha, 1913; Lindberg and Ernster, 1956), the techniques were tedious prior to 1920 and required large volumes of serum. In that year Bell and Doisy described a simple method for estimating blood and urine phosphate without preliminary ashing of the sample and extraction of ammonium phosphomolybdate and in 1921 Kramer and Tisdall described an oxalate precipitation - permanganate titration method for calcium in serum. Even today most phosphate methods are modifications of the Bell and Doisy method, while newer techniques for estimating calcium in biological materials are frequently compared with the Kramer-Tisdall method or one of its modifications. This method has been criticised on a number of occasions (MacIntyre, 1957), but these criticisms possibly arise from variations in the techniques used

for, as will be shown later, the Clark and Collip (1925) modification of the method was essentially sound and can give reliable results.

It was not long after the discovery of hypocalcaemia in cases of milk fever that an accompanying hypophosphataemia was also described (Fish, 1929; Sjollema, 1929). About the same time observations were made on the concentrations of calcium and phosphate in the serum of normal cows at calving but with little evidence of low values until it was realised that the changes in concentration were transient. These changes were eventually found to become progressively greater as cows age, but they do not occur together, as the hypophosphataemia is most marked within an hour or two of calving while the hypocalcaemia in normal cows is most severe during the first twenty-four hours after calving (Moodie, Marr and Robertson, 1955).

The state of the calcium in the blood has been the subject of a number of studies. The cells of ox blood are believed to contain variable small amounts of calcium but the bulk of the calcium is carried in the serum (Irving, 1957). The normal plasma or serum value lies between 8 and 12 mg. per 100 ml. and the calcium exists in two main forms - a diffusible ionised form which accounts for 3.6 to 7.7 mg. per 100 ml. (Hallgren, Carlstrom and Jonsson, 1959) and a non-diffusible protein bound portion. A small quantity of diffusible calcium is non-ionised and complexed with bicarbonate, phosphate and citrate (Neuman and Neuman, 1958).

The combination of calcium with serum protein can be split readily

when the serum calcium is lowered by dialysis or oxalate precipitation and similarly in cases of milk fever there is a reduction in both calcium ion and protein bound calcium concentrations. In some cases of hypocalcaemia the ionic concentration remains high although the bound calcium may fall and changes in the bound calcium do not seem to depend on changes in the serum protein levels (Hallgren et al., 1959). Vigue (1952), Larson and Kendall (1957) and Carlstrom (1961) have recorded lowering of the serum protein values at calving, but these affect α and β globulins principally and do not seem large enough to influence total serum calcium concentrations seriously. Carlstrom (1961), however, suggests that in milk fever the form of calcium binding, either to protein or some other hypothetical substance, is altered.

The total phosphate concentration of plasma normally ranges between ten and fourteen milligrams per cent and consists of lipid, ester and inorganic phosphates. The lipid phosphate concentration is quite variable between animals but tends to be specific for individuals and to fall slightly at calving. The ester phosphate in cattle plasma amounts to one or two milligrams per cent and the inorganic phosphate of adult cattle is normally three to seven milligrams per cent, depending on the diet. The inorganic phosphate as normally estimated includes some of the more labile esters such as phosphocreatine, acetyl-phosphate and ribose-1-phosphate but the amounts of these in normal bovine plasma are not known. Both inorganic and ester phosphate concentrations fall markedly at calving especially in older cows and the bulk of the change in total

plasma phosphate at this time can be attributed to the inorganic fraction (Moodie, Marr and Robertson, 1955).

Practically all the true inorganic phosphate of blood is believed to be ionised, but a very small proportion may be protein bound or complexed with divalent ions. Of the total orthophosphate in the serum, about one-fifth is in the form of H_2PO_4 ions and four-fifths as HPO_4 ions. PO_4 ions account for 0.008 per cent (Neuman and Neuman, 1958).

The concentrations of ionic calcium and phosphate in serum are such that it is undersaturated in respect of secondary calcium orthophosphate ($CaHPO_4.2H_2O$) which must form as a result of the activities of calcium and phosphate ions. This compound at the pH of the blood is unstable and soon hydrolyses to hydroxy apatite which has a much lower solubility in serum, so that in effect serum is supersaturated with respect to bone salt. It is only when the serum ionic calcium and ionic phosphate concentrations fall below certain values that bone can dissolve without the aid of local cellular reactions. It seems that dissolution of bone salt could occur when the product of the Ca^{++} and HPO_4^{--} concentrations, expressed as moles per litre, falls below 0.59×10^{-7}. This will occur, for example, with a Ca^{++} concentration of 4.0 mg. per cent and HPO_4^{--} of 1.9 mg. per cent, or of a Ca^{++} concentration of 6.0 mg. per cent and HPO_4^{--} concentration of 1.2 mg. per cent. This theoretical concept agrees with the view that total plasma calcium levels of about seven milligrams per cent can be maintained by simple equilibration

between blood and bone calcium (Hastings and Huggins, 1933; McLean and Urist, 1955; Copp, 1957).

The maintenance of normal serum calcium levels is believed to be primarily the function of the parathyroid glands and it was the similarity between the tetany of parathyroidectomy in dogs and the symptoms of milk fever which led Dryerre and Greig (1925) to postulate the existence of hypocalcaemia in this condition. Since that time the theory of parathyroid deficiency as the cause of parturient paresis has had its protagonists, despite the later observations that both calcium and inorganic phosphate concentrations are low in samples of blood from milk fever cases. At present the balance of evidence seems to be against the parathyroid deficiency theory. Jonsson (1960b) investigated parathyroid activity in cows and reviewed the literature relating thereto and has concluded that 'parturient paresis is not caused by parathyroid deficiency.'

Investigations based on parathyroidectomy in ruminants have always been subject to the criticism that some of the accessory glands may not have been removed. Stott and Smith (1957) thyro-parathyroidectomised five pregnant non-lactating cows and one non-pregnant lactating cow. Serum calcium levels dropped from an average of 10.3 mg. per cent to 7.2 mg. per cent on the second day after the operation, but the values returned to normal in three to five weeks. Todd, Fosgate, Cragle and Kamal (1962) obtained similar results for three cows in their second to third month of lactation. The plasma calcium and total phosphorus

concentrations averaged 10.0 and 10.6 mg. per cent post-operatively, compared with 9.8 and 10.4 mg. per cent before thyro-parathyroidectomy, and the effective removal of the glands was confirmed at autopsy. Payne (1963a) has thyro-parathyroidectomised twenty-nine goats which he subsequently maintained with thyroid hormone. The serum calcium fell within five to seven days to half normal values but stabilised between 7 and 8 mg. per cent in most animals for many months, which indicates the glands were completely removed. In two animals lower levels were recorded and these subsequently died. Hypophosphataemia also occurred, so the typical picture for non-ruminant species of an inverse relationship between serum calcium and phosphate was not observed in goats.

Administration of parathyroid extract to ruminants has produced variable reports. Jackson, Pappenhagen, Goetsch and Noller, (1962) attempted to restore normal plasma calcium levels in calving cows by administering parathyroid extract but did not observe any change in plasma calcium or inorganic phosphate levels. This result is in keeping with that reported by Lotz, Talmage and Comar (1954) for sheep. However, Todd et al., (1962) found that plasma calcium levels increased in parathyroidectomised cows following parathyroid extract administration but there was no significant change in plasma total phosphate although urinary phosphate excretion increased markedly. Sansom (1963) used isotope techniques on both normal and thyro-parathyroidectomised goats and suggested that parathyroid hormone may be important for the

absorption of calcium from the gastro-intestinal tract.

The stabilising level of 7-8 mg. per cent in Payne's thyro-parathyroidectomised goats is of some interest, in view of the aforementioned suggestion that this level can be maintained by the normal solubility of bone mineral in tissue fluids. Frequently this same value for calcium in the serum is taken as the borderline between 'normal' and 'clinically significant', when examining blood from a suspected milk fever case. The range of serum calcium values from clinical milk fever cases is about 3-7.5 mg. per cent which suggests that under certain circumstances the bone salt of older animals cannot dissolve at a rate sufficient to maintain higher levels.

This limitation may simply reflect the maximum rate of flow of blood through the spongy areas of the skeleton. Copp (1957) has estimated this flow in the dog as three to seven per cent of the cardiac output and considers that blood passing through the bones is only brought up to its normal serum calcium level. There is, however, evidence of diminished exchange between the circulating calcium and bone calcium in ageing cattle, (Hansard, Comar and Davis, 1954) but the extent to which this reflects a reduction in surface area of the skeleton or a reduction in the number or metabolic activity of the bone cells has yet to be established.

The solubility of bone salt is also influenced by the composition of its bathing fluid. Neuman and Neuman (1953) consider that hydrogen, sodium, magnesium, carbonate and citrate ions improve the solubility of hydroxy apatite and of these citrate is probably the most important.

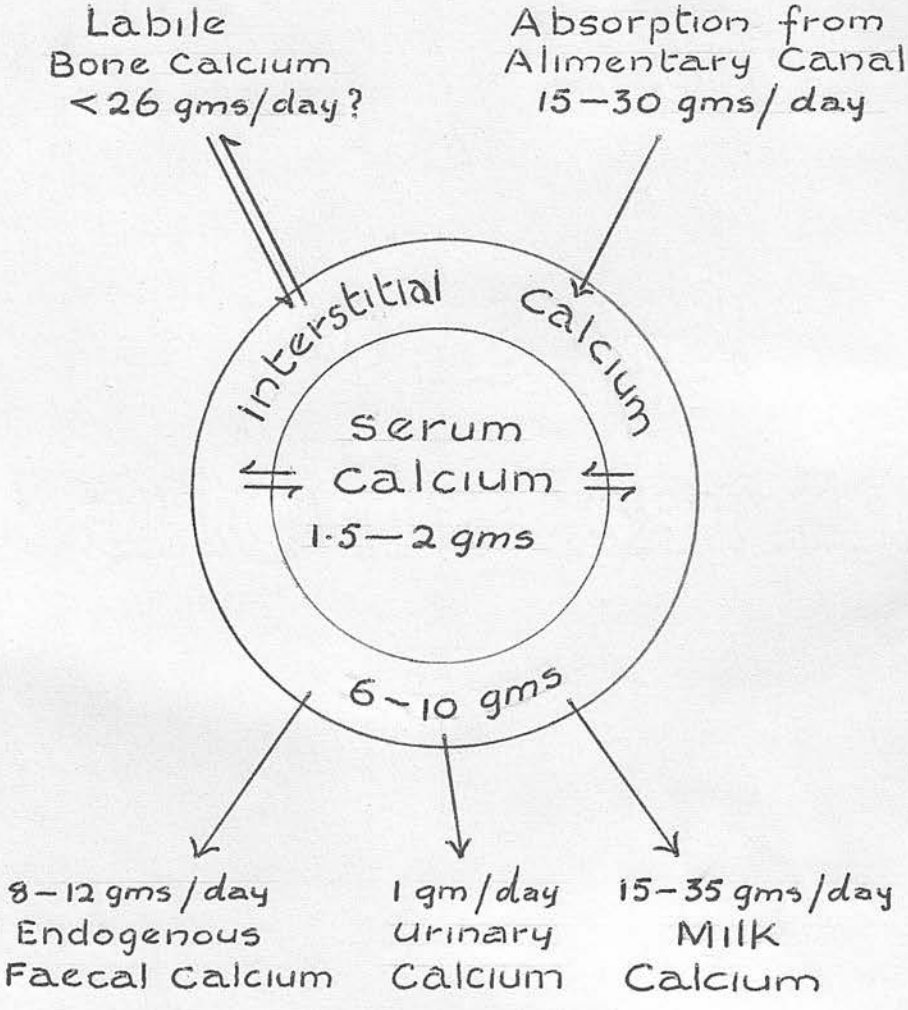
Citrate is normally found in high concentration in bone and is believed to be produced under the influence of the parathyroid hormone and vitamin D. Vitamin D also caused mobilisation of calcium from the alimentary tract of cattle (Conrad, Hansard and Hibbs, 1956).

The levels of citrate in the circulation, however, can be influenced not only by its release from the bones, but also by the metabolic processes in other parts of the body. Nevertheless, there have been reports of low levels of circulating citric acid in the blood of cows in association with hypocalcaemia soon after calving (Blosser and Smith, 1950), and Ward, Blosser, Adams and Crilly, (1953) have suggested that there may be some impairment of the Kreb's cycle in cows with milk fever, since blood lactic and pyruvic acids are elevated.

The turnover of calcium in the blood of a cow is very rapid and a quantity of calcium equal to the total amount in the circulation may be removed every one to five hours depending on the physiological state of the animal. The total circulating calcium is of the order of one and a half to two grams (based on a plasma volume of 4.5 per cent of body weight (Dale, Burge and Brody, 1957) and, as will be seen later, the daily turnover of calcium within the body can vary from about ten grams in the non-productive cow to over thirty-five grams in the lactating cow.

The blood can draw on a number of sources to maintain its calcium level (figure 1). The serum calcium is in equilibrium with the

FIGURE 1 The exchange of calcium between serum and tissues in lactating cows.



interstitial fluids and exchange between these two pools is considered to be very rapid, taking a few minutes at most. The total extracellular (interstitial plus serum) calcium is probably of the order of six to twelve grams. One dry Ayrshire cow carrying her second calf was given an intravenous injection of nineteen grams of sodium oxalate as a two per cent solution evenly over a period of fifty-five minutes and died when the serum calcium fell from 10.2 mg. per cent to 3.0 mg. per cent (see Section IV). The sodium oxalate would account for 5.6 g. of calcium, presumably drawn from the extracellular calcium pool, which indicates that the total pool in this animal was of the order of eight grams. Conversely, the intravenous injection of four ounces of calcium borogluconate solution containing approximately 8.4 g. calcium raised the serum calcium of twelve cows by 7.78 mg. per cent fifteen minutes after the injection (Moodie, Marr and Robertson, unpublished data). More recently, Payne (1963b) has calculated the immediately available calcium reserve to be six to ten grams. Luick, Boda and Kleiber (1957b) claim that a proportion of the bone calcium can also mix rapidly with the interstitial calcium, and estimate the extracellular plus this exchangeable bone calcium to total about one hundred and twenty grams. Such a high value, however, cannot be expected in all animals, since a reserve of this size and availability would prevent the development of hypocalcaemia.

Only very little calcium is to be found in the soft tissues of the body and the principle sources of supply to the blood are from the skeleton

and the alimentary tract. Skeletal calcium exists in two forms - a labile fraction, which Luick et al., (1957b) estimate at two to four kilograms (or 37 to 60 per cent of the total bone calcium) in four sterile Jersey heifers, and a stable fraction which is not available to the circulation. These workers calculated that in their animals the labile bone released about twenty-six grams of calcium per day. Hansard, Comar and Davis (1954), however, consider that the labile bone calcium in Herefords constituted only between six and twenty per cent of the total bone calcium in three year old cattle and between two and five per cent in thirteen year old cattle.

There is little evidence of any gross loss of calcium from the skeleton of animals over their productive life, as judged by carcass analysis, so any removal of labile calcium in times of negative balance must be replenished during periods of positive balance (Duncan, 1958). Milking cows during peak lactation, that is, when yielding more than about three gallons of milk per day, are believed to be in negative calcium balance and positive balances are recorded during late lactation.

The efficiency of absorption of calcium from the alimentary canal seems to vary according to age. Hansard, Comar and Plumlee (1954) in experiments with Hereford cattle came to the conclusion that the true digestibility of calcium decreases from 34 ± 8 per cent in mature animals to 22 ± 4 per cent in aged cattle. In dairy cattle Conrad, Hansard and Hibbs (1956) estimated lactating cows to have a true calcium digestibility of 44 per cent compared with a value of 33 per cent for

three aged dry dairy cows. In the latter experiment the improvement in calcium digestibility in lactating cows may not have been due to physiological factors since the dry and milking cows were fed markedly different rations with possibly quite different availabilities of calcium. However, a dairy cow's ration often contains forty to seventy grams of calcium per day of which fifteen to thirty grams may be absorbed, depending on age, appetite and type of feed.

Re-excretion of calcium through the gut forms a constant drain on the blood calcium. In normal adult cows it has been estimated as eight to twelve grams per day and does not seem to alter as the animal ages (Hansard et al., 1954; Conrad et al., 1956). This value may be a little high, as its calculation depends on the assumption that radioactive calcium added to the feed has the same availability as the natural calcium in the food. Visek, Monroe, Swanson and Comar (1953) used a different isotope technique and obtained values of five to nine grams per day for cows. Whatever the true value may be, in older animals where calcium absorption is failing, the constancy of the endogenous faecal calcium may lead to negative balances even in the non-productive cow. Loss of calcium through the urine accounts for up to one gram per day and urinary calcium output appears to decrease at parturition (Ward, Blosser and Adams, 1953).

The calcium content of the milk is about 0.15 per cent which means that every gallon produced removes about seven grams of calcium. Colostrum contains about the same amount of calcium except during the first day when the concentration may be higher (Garrett and Overman,

1940). Thus a cow secreting three gallons of milk or colostrum per day loses about twenty-one grams of calcium through the udder in addition to some ten grams endogenous faecal calcium and up to one gram of urinary calcium daily. If this loss from the circulation is not met in full by absorption from the digestive system, the balance of the animal's requirements will have to be obtained from the skeleton. In the absence of replenishment of the blood from the gut and the skeleton, one gallon colostrum would readily deplete the extracellular calcium pool to paretic levels.

The normal cow in milk is well able to maintain her serum calcium level, regardless of her age and state of nutrition, and there is some evidence that milk yield may in some way be balanced with calcium intake (Espe and Smith 1952). Therefore the majority of cases of hypocalcaemia in cattle cannot be explained unless a further factor is involved, such as a marked change in the calcium balance for which the animal cannot immediately compensate. Such a change could be the sudden need for calcium for milk secretion (which is greater than the preceeding need of the foetus, which is estimated at about 10 g. per day (Comar 1956)), interruption to the absorption of calcium from the gut (Marr, 1958), failure to mobilise calcium from the bones, or any combination of these in lactating animals. Urinary output and endogenous faecal losses do not appear to vary significantly and are unlikely to be of importance in precipitating acute imbalances.

Milk fever is most common in the newly calved cow, occurring mostly in older animals and in high producing strains of cows where the

output of milk is usually greater and where, as we have seen, the availability of bone and alimentary calcium may be lower. Since, however, the loss of calcium in the colostrum of cows developing milk fever is no greater than in normally calving old cows (Hibbs, Pounden and Krauss, 1951) and may even be less (Nilsson, 1960), this by itself does not account for the development of hypocalcaemia, especially when the loss of calcium into the colostrum in a number of cases would not appear to exceed a few grams at the time milk fever develops (for example, in cases which occur before calving). Consideration of these matters leads to the conclusion that the hypocalcaemia in the calving cow is essentially a failure to obtain sufficient calcium from the gut or the bone. Reference to the data given in figure 1 indicates that a cow secreting colostrum at three to four gallons per day could not provide for both milk and endogenous faecal calcium from either the skeleton or digestive system alone. An interruption in supply of even a few hours duration from one of these sources could have serious repercussions.

Little is known of the factors likely to arrest temporarily the mobilisation of calcium from bone, especially where serum levels are below the solubility product for bone salt, but constipation is a well-known feature of milk fever and is generally assumed to be the result of the hypocalcaemia, since the injection of calcium is frequently followed by evacuation of the bowel. However, this does not exclude the possibility that stasis of gut movement at the time of calving impairs the absorption of calcium and leads to a hypocalcaemia which, if it is

sufficiently severe, may in turn lead to further stasis of the digestive system. Although they did not draw attention to it, evidence of interference with alimentary activity is to be found in the paper by Ward et al., (1952) where feed and calcium intake remained normal over the calving period in heifers, but declined in older cows and in those which developed milk fever, and Rubenkov (1960) has reported slowed rumen movements in cows twelve to forty-eight hours before milk fever. Nilsson (1960) has observed loss of appetite and constipation in cows developing milk fever, prior to paresis.

The object of the work in this thesis is the furtherance of our knowledge of factors likely to precipitate acute changes in serum calcium concentration in the ruminant, particularly to values of less than seven milligrams per cent in cows at calving. The thesis is divided into a number of sections which include descriptions of special techniques developed for these investigations (section I), observations on the physiological and biochemical changes in cattle about the time of calving (sections II and III) and attempts to test the suggestion made in these sections that alimentary stasis may induce hypocalcaemia in milking cows (section IV). The prevention of hypocalcaemia is considered in section V.

Section VI describes experiments on sheep with the primary object of developing an arterio-venous technique for measuring the absorption of calcium and phosphate from the alimentary canal. Sections VII and VIII record observations to identify which hormones, if any, might influence calcium and phosphate metabolism at calving.

SECTION I

SPECIAL TECHNIQUES

Four special techniques were developed during the course of the work recorded in this thesis. These were:

(1) The recording of rumen movements in the intact cow;

(2) The collection of portal venous blood samples from conscious sheep;

(3) The collection of hepatic venous blood samples from conscious sheep; and

(4) The estimation of serum calcium with a high level of accuracy.

(1) The Recording of Rumen Movements in the Intact Cow

At the time the experiments were started only Dougherty and Crumb (1949) and Seren, Vacirca and Babini (1951) had reported methods for recording the rumen movements in intact animals. Since then Slanina (1957-58), Reid (1957) and Slanina and Gdovin (1963) have reported other methods, but only the latest reference gives a method which is simple enough for use under ordinary farm conditions. The method described here and reported by Alexander and Moodie in 1960 is simple, yet gives sensitive records of the movements of the dorsal sac of the rumen.

Method

The apparatus consisted of a balloon (9 cm. diameter) connected by a water manometer to a tambour. Pressure changes in the balloon were

FIGURE 2 Holder formed by two tin cans A and B soldered together, with rubber balloon C in position.

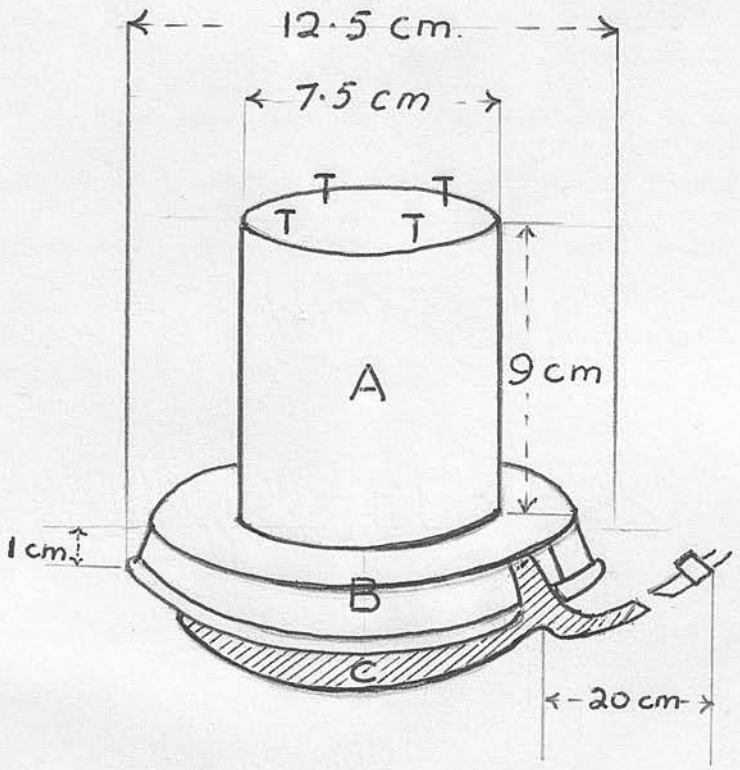
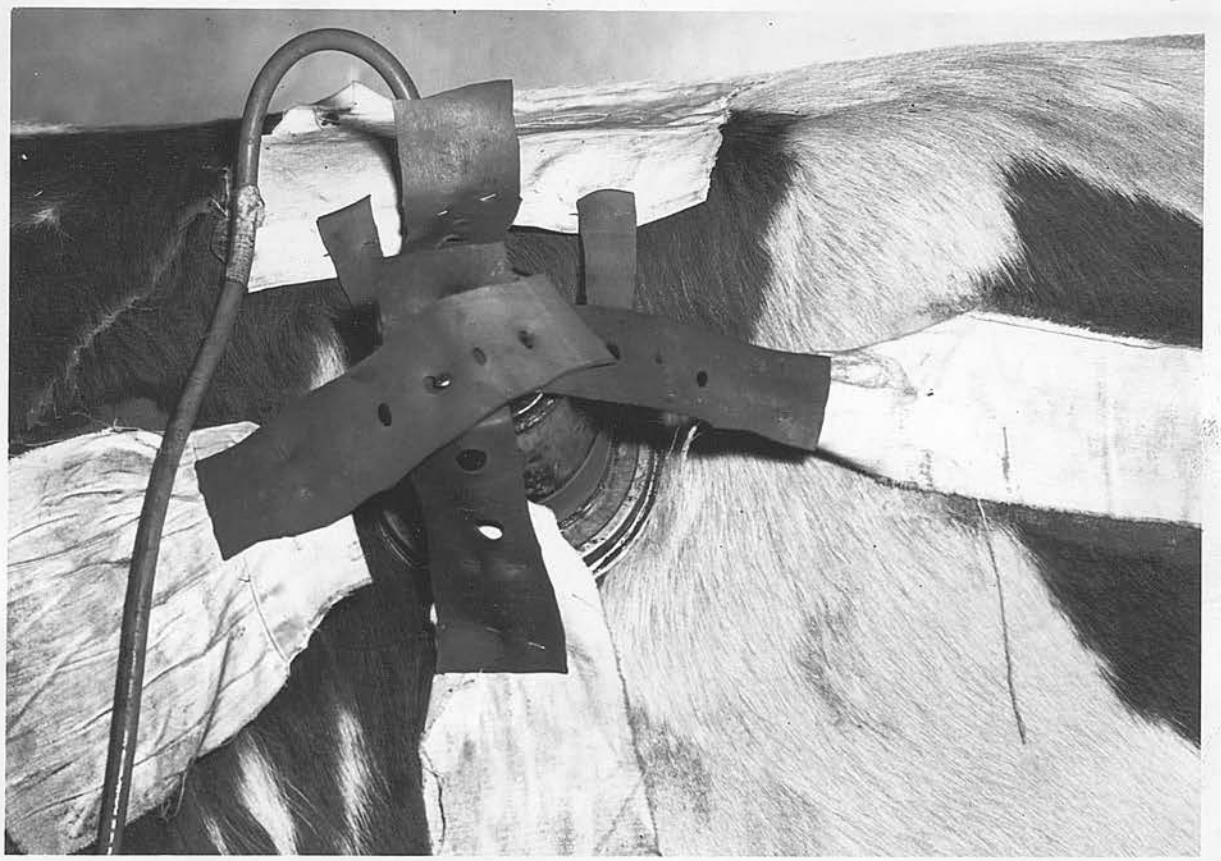


FIGURE 3 Holder attached to flank of cow, showing the position of the linen cloths on the animal.

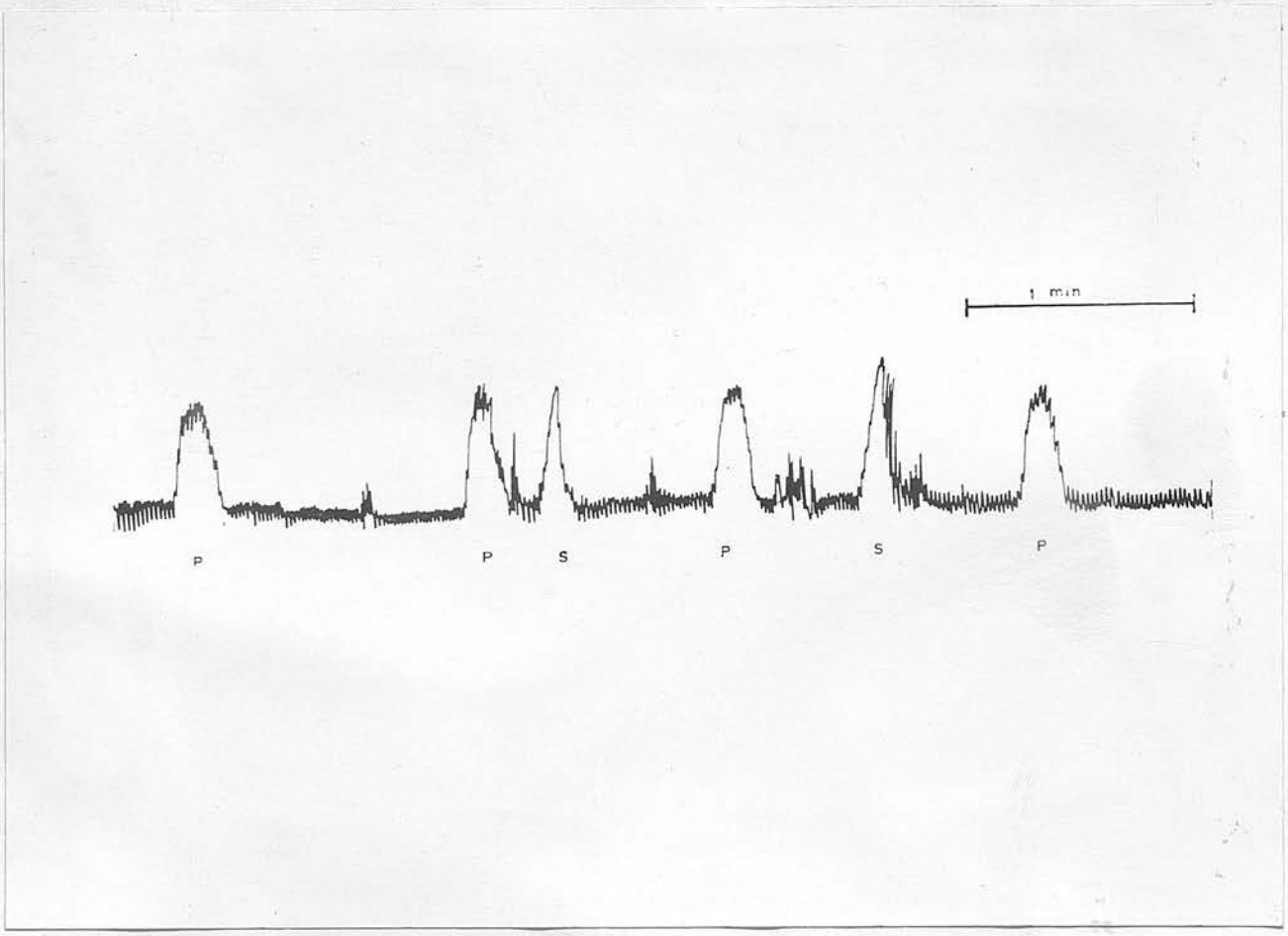


recorded kymographically with an ink writing point. The rubber balloon was arranged on the animal's left flank equidistant from the last rib, the extremities of the transverse lumbar processes and the iliac tuberosity, and was held in position by means of a holder made from tin cans (Figure 2) supported by a series of rubber straps attached to linen cloths which were in turn stuck to the skin of the subject by a suitable adhesive (Evostik, Evode Limited, Stafford) (Figure 3).

The subject was prepared a day before recordings were made. Four areas were clipped, scrubbed and shaved, after which the cow was left for several hours, since any small open wounds were found to be sensitive to the adhesive. Pieces of linen cloth were cut to fit the shaven areas, about five centimetres extra length being allowed on the inner ends. After preliminary cleansing of the skin with surgical spirit, the cloths and the shaven areas of the subject were treated with the adhesive and the cloths were applied to the cow leaving a five centimetre flap at the inner end of each cloth. These cloths remained in place for two or three weeks.

The balloon was attached to the holder by a centrally placed spot of glue and held in position at right angles to the flank by a series of four rubber straps each 15 x 4 x 0.15 cm., one radiating to each of the cloths. Attachment to the cloths was effected by safety pins. A further rubber strap of one centimetre width was looped around the holder and attached to the dorsal cloth to prevent the balloon from sliding down the flank.

FIGURE 4 Record of rumen movements of a normal cow showing primary (P) and secondary (S) waves.



primary (P) and secondary (S) waves as described by Stevens and Sellers (1959) were distinguished by their characteristic differences in form. The frequent small waves were due to respiratory movements and over a twelve minute tracing, twelve primary and seven secondary waves were observed.

The figure is almost identical to that obtained by Reid (1957) of pressure changes within the dorsal sac of the rumen and Dougherty and Crumb (1949) have already demonstrated the close similarity between simultaneous tracings made from two pressure recording devices, one internal and one external, in a fistulated cow. The frequency of eructation on the primary and secondary waves was also similar in the cows studied here and by Reid (1957) and Stevens and Sellers (1959).

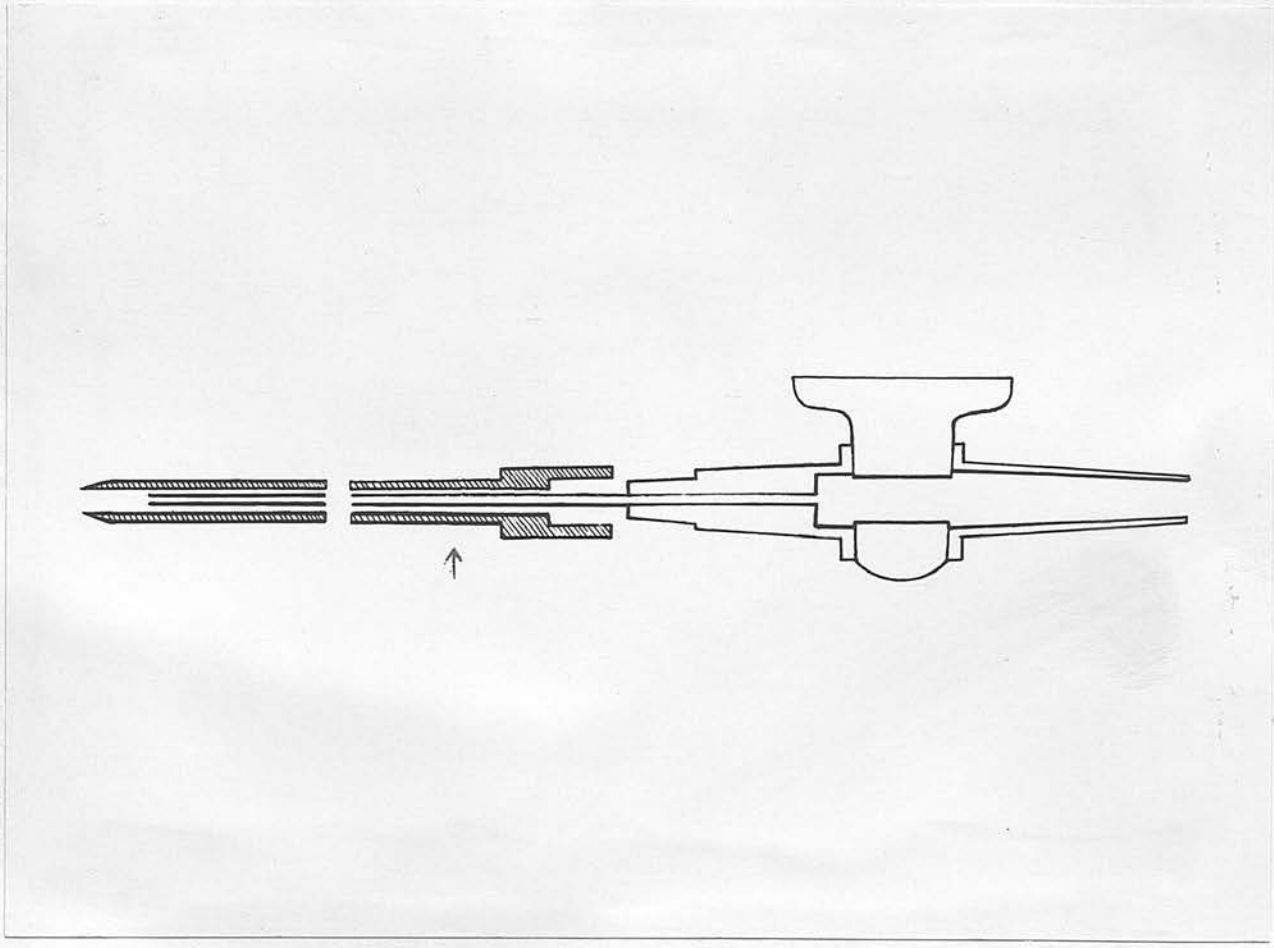
Summary

An apparatus is described which gives satisfactory recordings of the rumen movements of the intact cow.

(2) The Collection of Portal Venous Blood from Conscious Sheep

The estimation of the absorption of nutrients from the alimentary tract of conscious experimental animals by comparing the composition of portal venous and arterial bloods has been seriously hampered by the difficulty in maintaining portal catheters over prolonged periods. London (1935) described a method of applying a metal cannula to the wall of the portal vein and this is said to have proved satisfactory for sampling over a period of five months in a calf (Naumov, 1958) and a

FIGURE 5 Portal vein catheter



↑ Site for attachment of nylon tape used to hold the nylon tap in position.

and a modified London cannula has been used by Schambye (1951) in sheep. Denton, Gershoff and Elvehjem (1953), Jungblut, Lohmann, Schober and Turba (1955), Shoemaker, Walker and Van Itallie (1959) and Jackson, Radeleff and Buck (1960) described methods based on sampling from the smaller branches of the portal vein in the liver or on the passage of a catheter through a mesenteric vein to the porta hepatis. These have a short period of usefulness for blood sampling purposes (three weeks and frequently much less) and the catheters may become displaced. Conner and Fries (1960) have extended the period of use of such a catheter in a calf to seventy-three days but state that very large amounts of anticoagulants had to be used with possible serious side effects.

The method used here is simple and permits the withdrawal of portal blood rapidly, easily and with unlimited frequency over periods of several months, and only small amounts of anticoagulants were used.

Method

The catheter (Figure 5) consisted of two flexible grade polyamide (nylon) tubes * each 40 cm. long, one of which fitted inside the other. The external and internal diameters of the outer tube were 2.76 mm. and 1.90 mm. and of the inner 1.34 mm. and 1.00 mm. respectively. The inner tube had a nylon tap at its external end which fitted into a collar on the outer tube with a nylon tape to secure the fitting. The internal end of the outer tube was finely tapered without any reduction in bore.

* All nylon materials obtained from Portland Plastics Limited, Bassett House. Hythe. Kent. England.

Operative Procedure:- The animal was starved for twenty-four hours prior to operation and anaesthesia was induced and maintained with pentobarbitone sodium solution (65 mg./ml.). The jugular vein was cannulated with a nylon tube and an intravenous drip of normal saline containing five per cent glucose was established to facilitate the administration of maintenance doses of anaesthetic. With full aseptic precautions, the right flank was opened mid-way between the last rib and the coxal tuber, extending ventrally from two centimetres below the transverse lumbar processes for about thirty centimetres. The common mesentery was exposed and a large anterior branch of the intestinal trunk of the mesenteric vein freed from the accompanying artery and nerve for a length of about one centimetre. Two ligatures were passed around the vessel and that nearest to the small intestine tied off to arrest the flow of blood. A small incision was made in the vein between the ligatures and a pair of fine-pointed curved forceps inserted into the vessel in the direction of the blood flow. The outer tube of the catheter was passed in a forward direction between the points of the forceps, which were used to dilate the vein, until the tip lay in the portal vein at the entrance to the liver (porta hepatis). The second ligature was lightly tied to prevent bleeding between the vein and the tube, and the inner tube was inserted into the catheter, filled with anti-coagulant (1 ml. heparin solution, 600 i.u./ml.) and the tap closed. The position of the tip of the catheter was then confirmed by palpation and adjusted if necessary. A length of nylon tape (5 mm. width) was securely tied with the aid of nylon cement to the

catheter at its entrance to the vein, the tape and the catheter being first dried with ninety-five per cent ethyl alcohol. The ends of the tape, each of which was inserted twice through the common mesentery on each side of the tube, were loosely tied so as to draw the mesentery around the knot on the catheter. The catheter was led to the flank and the incision closed.

Maintenance of the Catheter:- The inner tube of the catheter was kept filled with heparin solution (600 i.u./ml.) and flushed with normal saline every eight hours during the first week, this being gradually reduced to twice daily at twelve hour intervals so long as the catheter remained patent. Crystalline penicillin, one million units in five millilitres normal saline, was flushed through the catheter twice daily during the first week, the last millilitre being mixed with one millilitre heparin solution to prevent clotting. Later this treatment was reduced to thrice weekly. The inner tube was removed weekly for cleaning, the outer tube thoroughly flushed with normal saline and the catheter checked for patency. It was not found necessary to withdraw blood each time the catheter was examined and provided fluid could be injected easily it appeared undesirable to tamper with the catheter especially during the first week after its insertion.

Sampling:- In favourable cases blood could be withdrawn by attaching a hypodermic syringe to the top of the inner tube. On other occasions the inner tube was removed and samples withdrawn through the outer tube, especially if it was suspected that a small clot was acting as a valve at the tip of the catheter. Usually, however, samples were most readily

TABLE I

Duration of patency of portal vein catheters

Sheep No.	Duration of patency (days)	Remarks
1	54	
2	112	
3	45	Died of septicaemia
4	112	
5	43	Catheter pulled out
6	86	
7	161	Died of multiple infarctions, catheter still patent
8	53	Catheter pulled out
Mean	83	

obtained by removing the internal tube and replacing it for the duration of an experiment with a slightly longer one so that its tip lay one to two centimetres beyond the end of the outer tube. The process of insertion was facilitated by stiffening the temporary tube with a length of 22 S.W.G. copper wire. On rare occasions it was necessary to clear debris from inside the outer tube and to break down obstructions from around its tip; this was done by passing a solid nylon rod of 1.5 mm. diameter several times through the outer tube.

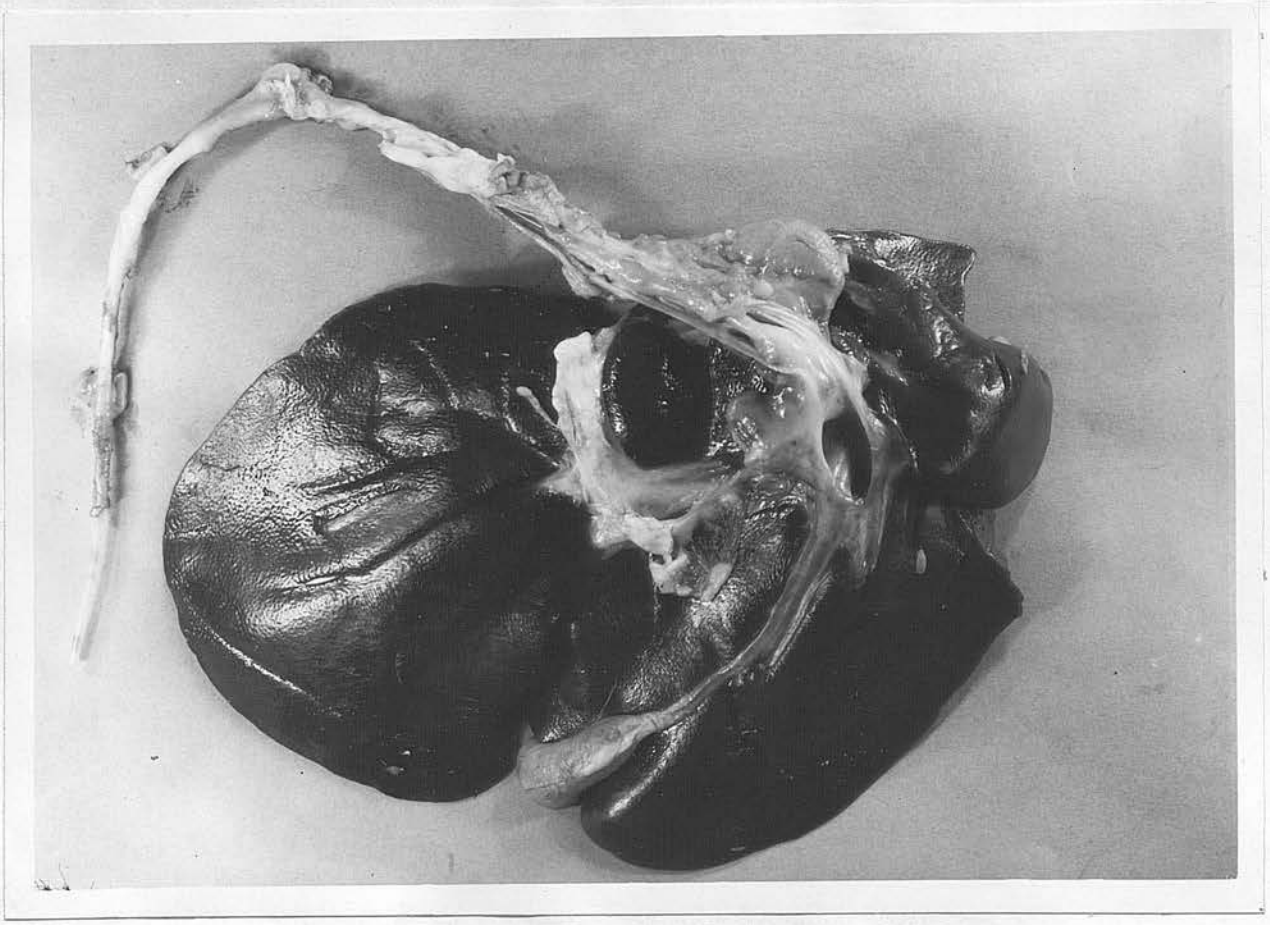
Results and Discussion

After major abdominal surgery a period of up to three weeks may elapse before alimentary activity, as shown by feeding habits and the characteristics of the faeces, can be regarded as normal. The techniques of Naumov (1958) and of Connor and Fries (1960) for calves permit a long recovery period, but the method described in this paper is probably simpler and the catheters had a mean period of usefulness of eighty-three days (range 43-161 days) (Table I). Two of the low values were caused by the catheters being pulled out inadvertently, probably by being caught on the pen walls, since in neither case had there been prior interference by the animal with the catheter nor any sign of discomfort. In both cases the outer tube had separated from the tape attaching it to the common mesentery, the tape being left in position, which emphasises the necessity for strong adhesion at this point. In more recent catheterisations the nylon tube has been roughened with a nail file at the site of attachment of the tape in order to strengthen

FIGURE 6 Intestinal vein and porta hepatis of a sheep ten days after catheterisation. The catheter with its fibrinous covering was lying free within the lumen of the intestinal vein and there was no evidence of obstruction to the flow of blood.

T - tip of catheter

FIGURE 7 Intestinal vein and porta hepatis of a sheep one hundred days after catheterisation. The catheter was embedded in the wall of the intestinal vein for most of its length and its tip was covered with tissue in the form of a papilla, which permitted injection of fluid but not withdrawal of blood samples. The intestinal vein was patent.



P - papilla of tissue over tip of catheter

C - catheter

the union.

The comparatively long life of these catheters was possibly due to a combination of factors. Nylon appears to be less reactive than the more usual polyvinylchloride and polyethylene catheters when used intravenously, although it becomes coated with a fibrinous deposit within half an hour of insertion. Subsequently a low grade phlebitis with intimal proliferation and medial hyperplasia develops. Figures 6 and 7 show the appearance of the catheter and the intestinal vein as seen at post-mortem ten days and one hundred days after catheter insertion. In the latter case the intestinal vein was still quite patent with the catheter firmly embedded in its wall. This is in contrast to two experiments using polyvinylchloride catheters which remained patent for only twenty-one and twenty-six days respectively and where complete occlusion of the intestinal vein occurred with gross enlargement of the veins of the intestinal arches.

The proliferation of the intima caused projections to form at the tip of the nylon catheter, and the development of papillae as shown in Figure 7. These conditions may lead to difficulty in withdrawing samples from a simple catheter, since they can obstruct the flow of blood when suction is applied although solutions can be infused easily. By using a double catheter with a longer inner tube these obstructions can be by-passed or penetrated; alternatively the obstructions at the tip of the catheter can be displaced with a solid rod as previously described.

The regular use of penicillin infused through the tube has proved

invaluable in controlling infection and even where its use has been withheld for some time and blood sampling has become impossible due to pus formation, complete restitution can occur following the reintroduction of penicillin infusions. In addition, the regular flushing of the tube with sterile saline and filling with heparin solution is especially important during the first week after the operation, when attention every eight hours is strongly recommended. Thereafter, regular twelve-hourly attention suffices unless difficulty is being experienced, when more frequent flushing is advantageous.

Summary

A portal vein catheter is described which remained patent for blood sampling purposes for an average of eighty-three days.

(3) The Collection of Mixed Hepatic Venous Blood from Conscious Sheep

The entry of the hepatic blood into the posterior vena cava is by means of a large number of veins, some of them small. This makes it impossible to collect a representative sample of hepatic venous blood by catheterisation of a hepatic vein, a technique which in any event requires general anaesthesia and x-ray facilities and so is not suitable for experimental large animals under field conditions. A mixed sample of hepatic venous blood may be obtained by the inflation of a balloon in the posterior vena cava between the hepatic and renal vessels, with subsequent sampling anterior to this point (Blalock and Mason, 1936), but this method must cause severe temporary changes in the blood circulation

and heart action with consequent changes in hepatic blood flow.

A more satisfactory solution would be the permanent occlusion of the posterior vena cava which would allow compensatory changes to take place. Chronic partial occlusion of the posterior vena cava has been carried out in connection with observations on ascites (McKee, Schilling, Tishkoff and Hyatt, 1949). Complete obstruction without obvious side effects has been observed in the sheep as a result of a thrombophlebitic lesion extending from a liver abscess (Head, 1958) and Ballinger, Haupt, Hering and Gibbon (1959) produced the corresponding occlusion in the dog following the formation of a caval-azygos shunt which subsequently thrombosed.

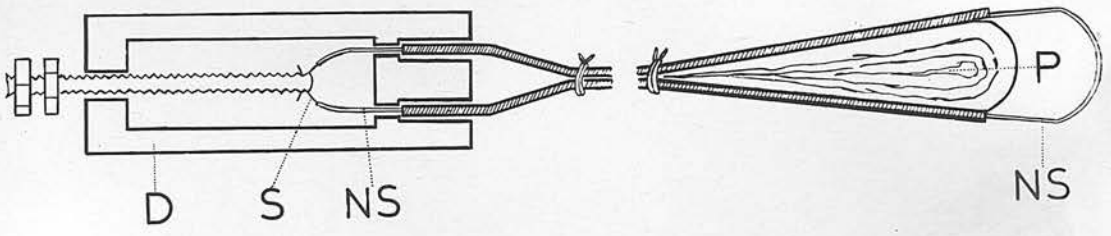
The ligation of the posterior vena cava between the renal and hepatic vessels as described below permits the collection of hepatic venous blood over periods of at least a year. The technique consists of attaching a snare around the posterior vena cava between the renal and hepatic vessels. After gradual occlusion of this vessel mixed hepatic venous blood can be obtained by passing a catheter from the jugular vein into the posterior vena cava.

Method

The snare (Figure 8) consisted of two fifteen centimetre lengths of flexible grade nylon tubing of 1.5 mm. bore and 2.1 mm. external diameter and one longer length of 1.0 mm. bore. These three tubes were bound together at intervals of about two centimetres with inert suture material (Supramid Braun), * and at one end a pad of about one

* B. Braun, Melsungen, Germany.

FIGURE 8 Snare for occluding the posterior vena cava



D - duralum rod

NS - nylon covered stainless steel snare

S - stainless steel core of snare

P - pad

centimetre diameter was inserted between the main tubes. The pad was made by doubling over an eight centimetre length of one centimetre autoclave tubing lightly packed with cotton wool, the ends of which were sealed with nylon cement.

A sixty centimetre length of 27 S.W.G. stainless steel wire was threaded through a shorter (about forty-five centimetre) length of nylon tubing (external diameter 0.94 mm. bore 0.75 mm.). The covered wire was then inserted through the main tubes to form a loop over the pad, thus forming the snare. To the other end of the device was fitted a tightening screw consisting of a duralum rod 4.5 cm. long and 1.5 cm. diameter, with a 2.5 cm. window towards one end. The base was drilled right through into the window with two holes large enough to allow the stainless steel wire and its nylon covering to pass through, these holes being enlarged for three-quarters of their depth to neatly fit the main supporting tubes of the shaft of the snare. The other end of the duralum fitting was drilled to take a 3.5 cm. threaded rod. This rod had two locking nuts at its outer end and a hole at its inner end allowing two strands of the stainless steel wire to pass through. With the snare drawn up tightly against the pad, the nylon covering of the stainless steel wire was cut back on both ends to a point about five millimetres short of the threaded-rod end of the window. Finally the third nylon tube of the shaft was shortened to the overall length of the appliance.

Operative Procedure - Stage I:- The sheep was prepared and anaesthetised as described above (page 20), except that the preparation

of the right flank was centred on the margin of the last right rib, extending from the spinous processes of the lumbar vertebrae ventrally for forty centimetres. A twenty-five centimetre paracostal incision was made as close to the last rib as possible, commencing high in the angle between the last rib and the lumbar transverse processes. Care was taken not to incise the parietal peritoneum, which was separated from the overlying tissues in an antero-medial direction until the midline was reached. The peritoneum was ruptured at this stage to expose the edge of the liver at the point of entry of the posterior vena cava, a pair of large curved forceps was passed between the vessel and the muscles of the back immediately posterior to the liver and a long length of suture material drawn through.

The snare was then inserted without its duralum attachment and with the stainless steel wire threaded through only one tube of the shaft. The long end of this wire was attached to the thread, carefully drawn behind the vein and then passed through the second tube of the shaft of the snare. The wires were drawn up until both ends of the nylon covering to the wire became visible and the incision was then closed.

The duralum fitting was fastened to the main tubes of the snare with adhesive, the threaded rod inserted, the ends of the stainless steel wire passed through in opposite directions, pulled up as far as their nylon coverings would allow and twisted together. This ensured that the loop of the snare would automatically be drawn tight on to its pad when the snare was closed. Finally nuts were fixed to the threaded

FIGURE 9 Sheep with snare in position during tightening-up period.

FIGURE 10 Close-up of Figure 9.

rod and locked in position, leaving the snare fully open.

Stage II:- The loop of the snare was reduced by tightening the nuts over a period of several weeks. This was done in approximately six stages at five day intervals. For a few days after the operation small quantities of crystalline penicillin solution (one millilitre containing 200,000 units) were injected down the third tube of the shaft and repeated later as necessary. Figures 9 and 10 show sheep with the snare in position.

Stage III:- After closure of the snare the external portion of the apparatus was removed, although it was found that this could be left for many weeks without apparent discomfort to the sheep. Under general anaesthesia and with aseptic precautions, the original operation site was re-opened, the shaft of the snare shortened by about five centimetres and the stainless steel wires exposed, pulled up tight and twisted together, thus permanently tightening the snare around the posterior vena cava.

The ends of the wire were folded back and bound with inert suture material and the incision closed over the snare.

Sampling:- This was carried out by passing a catheter from the jugular vein through the right atrium into the posterior vena cava, in either anaesthetised or non-anaesthetised sheep. A sixty centimetre catheter of 1.34 mm. external diameter and 1.00 mm. bore nylon tubing was suitable, this being stiffened with a 22 S.W.G. copper stilette having a gentle curve. A 13 B.W.G. serum needle was inserted under local anaesthesia into the jugular vein and the catheter passed through

it into the heart and with gentle manipulation into the posterior vena cava. In large sheep an obstruction was met at an insertion depth of about forty-five centimetres possibly due to the valves of hepatic veins entering the posterior vena cava close to the diaphragmatic foramen. The 13 B.W.G. needle was then removed over the outer end of the catheter and sampling carried out by means of a hypodermic syringe and needle attached to the catheter. Between samples the stilette was replaced to maintain the catheter in position and the end of the stilette was tied to a string about the animal's neck.

Results and Discussion

Preliminary experiments were carried out to determine the most satisfactory method of occluding the posterior vena cava. Ligation posterior to the renal veins was satisfactory in one sheep but ligation anterior to these vessels caused death in about ten days associated with ascites and uraemia in two sheep while in a third the vena cava ruptured soon after the operation. A more gradual ligation was clearly necessary and the snare described in this paper is a development of the technique of Verney (1960) for temporary occlusion of the adrenal vessels in the conscious dog.

The method described here has been used on six animals, four of which have subsequently been examined post-mortem. In three the vein was completely occluded, but in the fourth, where the snare was completely removed at Stage III of the operation, the vein was patent although constricted.

TABLE II

Examples of the appearance of dye in the posterior vena cava and heart of normal and ligated sheep following the injection of 100 mg. sodium bromosulphthalein in ten seconds into the saphenous vein.

	Site of Catheter	Dye concentration (absorptiometer readings x 10^3) Time from commencement of injection (sec.)			
		5-10	15-20	25-30	35-40
Normal Sheep	Heart	410	335	780	695
	P.V.C.	2000 +	1220	795	740
Partial occlusion of vena cava	Heart	195	95	166	461
	P.V.C.	492	2000 +	1050	700
	Heart	0	2000 +	1365	910
	P.V.C.	0	149	180	730
Complete occlusion of vena cava	Heart	0	1120	780	510
	P.V.C. *	0	112	150	190
	Heart	0	1540	2000 +	835
	P.V.C.	0	0	0	52
	Heart	0	0	455	1120
	P.V.C.	0	0	0	0
	Heart	0	2000 +	2000 +	1900
	P.V.C.	0	0	0	178

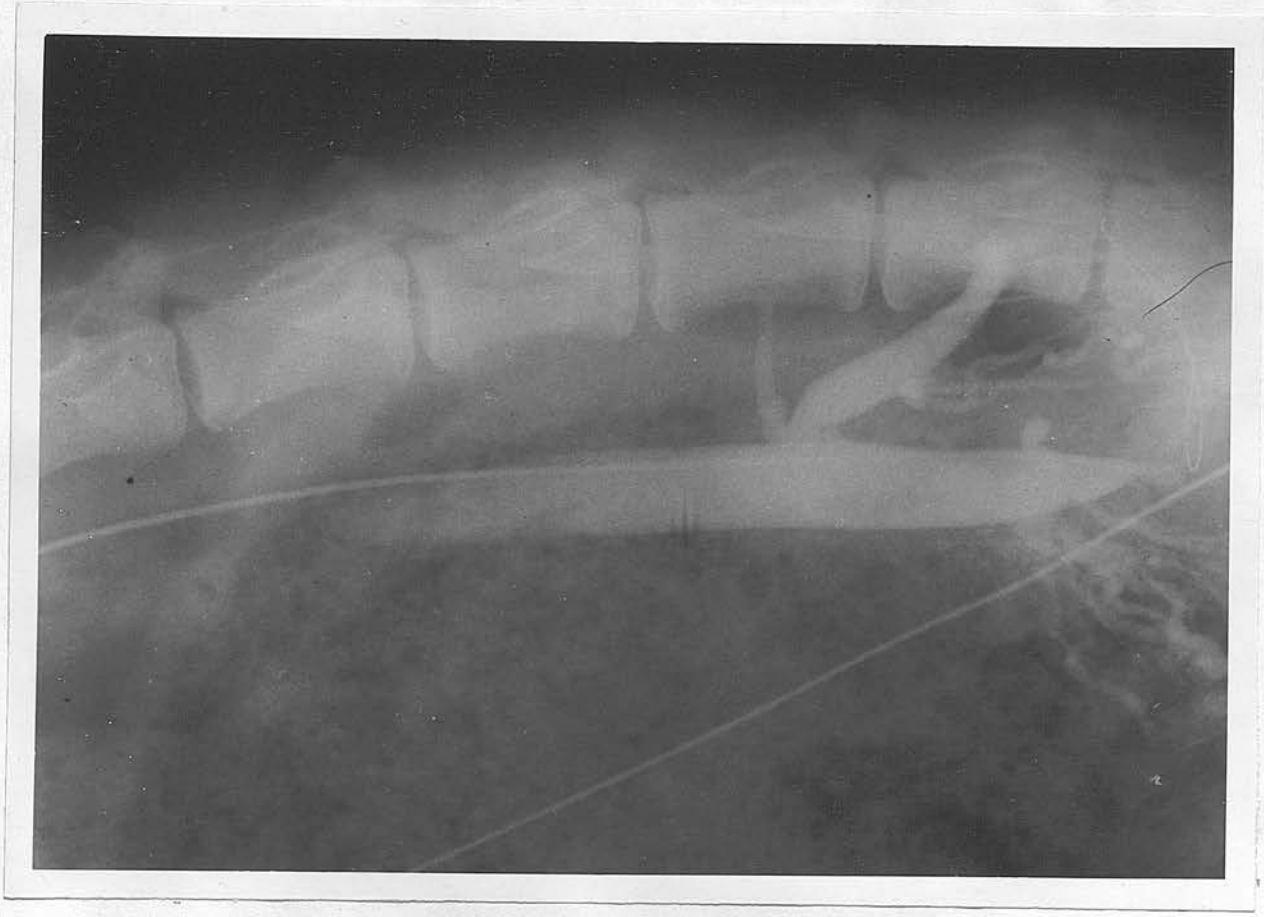
* Catheter incompletely inserted.

P.V.C. = Posterior vena cava.

The tightening of the snare in these experiments was carried out over a period of about a month, but it is possible that a shorter time could be adopted. Overtightening can be detected by clinical and biochemical examination for uraemia and in most cases a longer interval before the next adjustment allows the circulation to compensate. If necessary the tension can be released and the wires forced back down the shaft of the snare. This was not necessary in any of these experiments but it was for this reason that a wire snare was used in preference to less rigid materials. It is important, however, that the material used should not stretch under tension (copper wire is unsuitable).

The posterior vena cava was tested for occlusion by a simple dye infusion technique. The animal was anaesthetised, placed on a table, a catheter passed into the posterior vena cava in the region of the hepatic vessels and another into the heart. Sodium bromo-sulphthalein, 100 mg. in 5 ml. water was injected into a saphenous vein over a period of ten seconds and blood samples collected from the catheters five to ten, fifteen to twenty, twenty-five to thirty and thirty-five to forty seconds from the commencement of the infusion. The dye appeared simultaneously in the posterior vena cava and the heart in normal sheep and also where the vein was incompletely occluded or the caval catheter incompletely inserted; but where the vena cava was efficiently sealed there was a twenty second difference in the time of appearance of the dye at the two sites (Table II). The procedure was repeated three or four times as a precaution against erroneous results being obtained due to the tip of the hepatic catheter lying within a hepatic vein.

FIGURE 11 X-ray of a sheep with ligated posterior vena cava, during an injection of 45 per cent w/v sodium diatrizoate at the rate of three millilitres per second through a catheter passed as far forward as possible into the vena cava from the saphenous vein.



C - catheter

T - tip of catheter

S - snare

FIGURE 11 X-ray of a sheep with ligated posterior vena cava, during an injection of 45 per cent w/v sodium diatrizoate at the rate of three millilitres per second through a catheter passed as far forward as possible into the vena cava from the saphenous vein.

C - catheter

T - tip of catheter

S - snare

With too shallow an insertion the blood sample may be contaminated by heart blood passing back into the posterior vena cava.

A more reliable test can be obtained by passing a catheter through a femoral or saphenous vein into the posterior vena cava and probing gently. If the vein is patent, the catheter will be found to pass forward beyond the position of the snare. A further test is to inject a radio-opaque material through this catheter when it is inserted as far as possible. Figure 11 shows an x-ray of a ligated sheep four to six seconds after the commencement of the injection of forty-five per cent w/v sodium diatrizoate * at the rate of three millilitres per second. The reversal of the blood flow in the vena cava posterior to the snare is clearly shown.

The exact route taken by the blood passing from the kidneys and hind-quarters to the heart in ligated sheep is not clear. P.H. Hutton of the Department of Veterinary Anatomy of the Royal (Dick) School of Veterinary Studies has carefully dissected three of these sheep and concluded that the return is by a generalised increase in blood flow through all the normal venous anastomoses. Figure 11 indicates that the common lumbar trunk, anastomosing with the vena azygos, may be carrying an increased flow.

This method of preparing animals for sampling hepatic venous blood requires time for reasonable circulatory compensation. Edwards (1958) considered that in the human the blood pressure below a vena caval occlusion may remain elevated for four years, but the corresponding data

* Bayer Products, Surbiton-on-Thames, England.

for sheep have not been obtained. However, the useful life of animals prepared in this manner may be quite considerable, since two sheep which were allowed to survive for eight months remained perfectly healthy and the method has the advantage that sampling can be carried out at any time by catheterisation of the non-anaesthetised animal.

Summary

A method is described for collecting mixed hepatic venous blood from sheep. The technique involves permanent ligation of the posterior vena cava between the renal and hepatic veins and the catheterisation of the anterior portion of the posterior vena cava from the jugular vein.

(4) The Estimation of Serum Calcium

Sections VI and VIII describe experiments which involve the determination of the net absorption of calcium from the digestive tract of sheep by observing the changes in the composition of blood traversing the region. At the outset of these experiments calculations, based on a daily plasma flow through the splanchnic area of one thousand litres per day (700 ml./min.) and a daily net absorption of two grams of calcium, indicated that the difference in calcium concentration might be of the order of 0.2 mg. per 100 ml. plasma. A method with a much better reproducability than this was clearly necessary and steps were taken to standardise and if necessary to modify the methods in use in the laboratory at that time, namely, the oxalate precipitation and permanganate titration method of Clark and Collip (1925).

Method

After many trials the following method was adopted for heparinised plasma.

One millilitre aliquots of plasma dispensed with a syringe pipette * were transferred into 100 x 12 mm. round-bottomed centrifuge tubes, graduated at 5 ml. containing 1 ml. distilled water and 0.5 ml. 4 per cent ammonium oxalate solution. The contents of the tube were stirred with a fine glass rod and allowed to stand for thirty minutes. The tubes were centrifuged for five minutes (3,000 g.), the supernatant fluid decanted and the tubes drained for about a minute. The inside of each tube for about half its length was dried with a roll of filter paper and then rubbed with a rubber-ended stirring rod, moistened with a wash solution of dilute ammonia (2 ml. ammonia (sp. gr. 0.88) in 98 ml. distilled water), to remove all traces of oxalate from the rim of the tube. The wash solution was run down inside the tubes to a total volume of five millilitres and at the same time the tubes were shaken to disperse the precipitates. After centrifuging for three minutes (3,000 g.) the tubes were again drained and dried. Within an hour of titration, one millilitre of 2N sulphuric acid was added to each tube, and the tubes placed in a water bath thermostatically controlled at 90°C. About eighty per cent of the total expected volume of N/200 potassium permanganate was injected into the hot oxalate-sulphuric acid mixture with an automatic pipette * and the titration completed rapidly, using a microburette.

* B.D. Cornwall Pipettes, Shandon Scientific Company Limited, 9 Cromwell Place, London, S.W.7.

Readings were judged to the third decimal place in a total titration volume of about one millilitre. Blanks were taken through the method and the appropriate adjustment made to all calculations.

Calcium was estimated on heparinised whole blood as follows:-

Two millilitres of blood were added to 8 ml. of ten per cent trichloroacetic acid, mixed thoroughly and centrifuged or filtered after five minutes. Five millilitres of the supernatant or filtrate was transferred to a round-bottomed centrifuge tube as used for the plasma estimation and neutralised with concentrated ammonia and dilute acetic acid to a faintly pink end point using phenolphthalein indicator. One millilitre of four per cent sodium oxalate was added and the estimation completed as for plasma.

Results and Discussion

Numerous modifications of the original Kramer and Tisdall (1921) method have been proposed from time to time to enhance its reliability but the method described here is basically that of Clark and Collip (1925). The modifications proposed by other workers probably reflect a failure to observe certain important points of detail and in fact very slight deviations from the method can markedly influence the final result. Where a high degree of accuracy is required it is important that sources of error be either eliminated or, if this is impossible, standardised.

The quantities of plasma, water and oxalate used for the initial precipitation were half those used in the original method. This economy in the use of plasma has the advantage of facilitating the subsequent washing and as the proportions of the reagents remained constant the

efficiency of precipitation was not impaired.

Although not all the calcium present is likely to be precipitated in thirty minutes, extension of the precipitation time may lead to excessive deposition of calcium-magnesium-oxalate complexes (MacIntyre, 1957) and of some protein (Smith, Craig, Bird, Boyle, Iseri, Jacobson and Myers, 1950). The presence of protein can be exceedingly troublesome at the final titration and is best avoided. Consequently a standard time of thirty minutes was set.

Some of the 'calcium' recovered in these experiments may represent residual ammonium oxalate and the quantity must be standardised in the draining and washing of the tubes. The insolubility of calcium oxalate in the two per cent ammonia wash solution is dependent on the presence of some ammonium oxalate and a second wash with ammonia removes calcium oxalate equivalent to about one milligram calcium per cent. This could account for the high losses reported by MacIntyre (1957). Even in the first washing the calcium oxalate is not completely insoluble and this stage should be completed as rapidly as possible. Simple rinsing of the tube with the wash solution was found to leave significant and variable amounts of ammonium oxalate adhering to the sides, especially towards the lip, but rubbing down the sides of the tubes as described above removed this source of error.

The potassium permanganate titration must also be standardised. N/200 potassium permanganate is sufficiently coloured to give a good end point but it is significantly decomposed by light and heat, so a slow

Readings were judged to the third decimal place in a total titration volume of about one millilitre. Blanks were taken through the method and the appropriate adjustment made to all calculations.

Calcium was estimated on heparinised whole blood as follows:-

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Results and Discussion

Numerous modifications of the original Kramer and Tisdall (1921) method have been proposed from time to time to enhance its reliability but the method described here is basically that of Clark and Collip (1925). The modifications proposed by other workers probably reflect a failure to observe certain important points of detail and in fact very slight deviations from the method can markedly influence the final result. Where a high degree of accuracy is required it is important that sources of error be either eliminated or, if this is impossible, standardised.

The quantities of plasma, water and oxalate used for the initial precipitation were half those used in the original method. This economy in the use of plasma has the advantage of facilitating the subsequent washing and as the proportions of the reagents remained constant the

TABLE III

The recovery of standard calcium chloride solution added to samples of sheep plasma and blood

mg./100 ml.

Sample	0.9 ml. Plasma or Blood +0.1 ml. Water			0.9 ml. Plasma or Blood +0.1 ml. Standard Solution (4 mg.Ca/100 ml.)			Percentage Recovery
	No. of Replicates	Mean	S.E.	No. of Replicates	Mean	S.E.	
Plasma 1) direct	12	8.54	0.006	10	12.45	0.007	97.8
2) precipitation	9	9.11	0.030	9	13.08	0.020	99.3
3)	11	8.46	0.015	12	12.45	0.018	99.8
Blood 1)	3	7.52	0.029	3	11.26	0.006	93.5
2)	4	7.05	0.021	4	11.03	0.065	99.5
3) trichloro-	4	6.76	0.022	4	10.79	0.028	100.8
4) acetic acid	3	7.97	0.025	3	11.73	0.047	94.0
5) filtrate	4	7.75	0.024	4	11.65	0.039	97.5
6)	4	7.89	0.029	4	11.83	0.030	98.5

TABLE IV

Replicate estimates of calcium in sheep plasma and blood

mg./100 ml.

Sample	No. of Replicates	Mean	S.D.
Plasma 1	32	11.80	0.050
Plasma 2a	15	10.54	0.025
Plasma 2b	16	10.58	0.038
Plasma 2c	12	10.54	0.041
Plasma 3	15	10.97	0.026
Plasma 4	16	11.01	0.035
Plasma 5	13	10.53	0.041
Blood 1	29	7.36	0.075
Blood 2	27	7.54	0.085
Blood 3	27	9.21	0.109

Plasma Sample 2 was estimated as three successive batches - 2a, 2b and 2c.

TABLE V

Precision of serum or plasma calcium estimation by various methods as reported in the literature.

Method	Reference	Recovery of calcium added to serum or plasma (per cent)	Range or S.D. of replicate estimations mg./100 ml.
Oxalate pptn. followed by:-			
Permanganate titration	Clark and Collip (1925)	99.9	Range 0.2
E.D.T.A. titration	Wilson (1955)	99.2 (95 - 102)	S.D.-(0.08; 0.08; (0.08; 0.19; (0.19; 0.21.
Flame photometric analysis	Powell (1953)	98.4 (90 - 108)	-
	Llaurado (1954)	100	'Perfect' - results to nearest 0.1 mg. per cent.
	MacIntyre (1957)	99.8 (98.3 - 101.2)	S.E. of difference between duplicates = 0.12
Direct estimation:-			
2, 3, 4 - trihydroxybenzoic acid	Tsao (1952)	93.9 (92 - 95.5)	S.D. - 0.17; 0.20; 0.20.
Flame photometer	Winer and Kuhns (1953)	101.4 (100 - 104)	-
E.D.T.A. spectrophotometrically	Fales (1953)	96; 104	S.D. - 0.03
E.D.T.A. with Calcon	Pappenhagen and Jackson (1960)	-	Range 0.1
Ammoniacal zinc - E.D.T.A. (polarograph)	Irving and Watts (1961)	-	S.D. - 0.22

of standard calcium chloride added to plasma, shown in Table III, was between 97.8 and 99.8 per cent. Replicate estimates of the calcium in five samples of plasma are shown in Table IV and the standard deviations of the replicates ranged between 0.025 and 0.050 mg. per cent. These standard deviations are very little greater than would be expected from titration alone, since the titration of N/200 potassium permanganate against one millilitre of N/200 sodium oxalate was found to have a standard deviation equivalent to 0.037 mg. Ca. per cent.

These results may be compared with those previously reported in the literature for this and other methods where similar information is available (Table V). In only one case were perfect recoveries claimed, but here only a single short series of results given to the nearest 0.1 mg. per cent was reported. The Table shows that most methods give a good average recovery of calcium added to serum or plasma but considerable variation is reported in some cases. The standard deviations of replicate estimations quoted by these authors were considerably higher than with the method being described here, with the exception of the ethylenediamine tetra-acetate methods of Pappenhagen and Jackson (1960) and Fales (1953).

The estimation of calcium on whole blood is not commonly attempted but was necessary for the experiments described in Section VIII. Direct precipitation from laked blood resulted in the formation of a gelatinous mass which made washing impossible, but Sendroy (1944) has shown that a trichloroacetic acid extraction procedure is very satisfactory for plasma. Tables III and IV show the results of a similar extraction

procedure applied to heparinised blood. The recovery rate of added calcium and the reproducability of the technique are not so good as with the direct precipitation of calcium from plasma.

The Kramer-Tisdall procedure is frequently considered to be laborious, but this is only because two and sometimes three washes of the precipitate have been considered necessary (Kramer and Tisdall, 1921; Tisdall, 1923). In fact the method as described here is very convenient where other estimations are being carried out simultaneously since it is possible to arrange breaks in the flow of the work. One person can complete forty-eight estimations from the initial plasma stage in about three hours, of which only two represent active work. There is little operator fatigue since each stage of the work only lasts thirty to forty-five minutes on batches of this size. These factors, together with the recovery rates and the relative simplicity of the method, indicate that the technique compares favourably with other methods, except in those cases where an immediate result is required.

Summary

An oxalate precipitation, permanganate titration method for estimating plasma and blood calcium is described. The recovery of calcium added to plasma and blood was 97.8 to 99.8 per cent and 93.5 to 100.8 per cent respectively, and the standard deviations of replicate estimations were 0.025 to 0.050 mg. per cent and 0.075 to 0.109 mg. per cent respectively.

SECTION II

PHYSIOLOGICAL OBSERVATIONS ON COWS AT CALVING

The importance of cows having a continuous absorption of calcium from the alimentary canal was discussed in the introduction to this thesis, but apparently no observations have been made on the physiological activity of the alimentary tract at calving time. As a preliminary step in assessing the place, if any, of alimentary inactivity in the aetiology of parturient hypocalcaemia, the daily food intake and mineral consumption of a number of calving cows was recorded. Later, when the work appeared to be progressing favourably, the observations were extended to include rumen activity and faecal output. Milk yield data were also collected.

Materials and Methods

Animals:- Observations were made over the calving period on ten Ayrshire cows belonging to the Veterinary Field Station of the Royal (Dick) School of Veterinary Studies. The animals were selected according to age and were grouped into young and old cows as follows:-

Young cows - two heifers, one second calving and two third calving cows (cows 1a and 1b, 2, and 3a and 3b respectively),

Old cows - two fourth calving, two fifth calving and one eighth calving cow (cows 4 and 4M, 5 and 5MF, and 8).

All the calvings were normal except for one fourth calving cow, 4M, in which clinical metritis developed on the second day after calving, and one fifth calving cow, 5MF, which developed milk fever twelve hours post-partum. Data from the cow with metritis has been excluded from all averages and calculations based on these averages, but that from the milk fever cow was included. The young cows were all under 5.2 years old and the old cows over 5.9 years old.

Feeding:- The cows were fed the normal rations for the season of the year, except in summer when they were kept in stalls and fed on hay and concentrates instead of being turned out to grass. The chosen ration was fed for at least seven days before the commencement of the observations, during which time an estimate of the daily food intake was made. Bags were then filled with known amounts of food, a sufficient number being prepared to last the animal until after calving, and samples were taken for analyses of their moisture, calcium and phosphorus contents. The cows were fed from these bags for a minimum of four days before calving, residues being collected and analysed for moisture content. The calcium and phosphorus contents of the residues were taken as being the same as in the original food.

The concentrates normally consisted of dairy cubes, sometimes supplemented with a home mixed ration containing oats, high protein dairy cubes and soya cake. The total quantity of concentrates offered varied from six to fourteen pounds per day depending on the expected milk yield of the cow. Roots, when available, were fed at the rate of

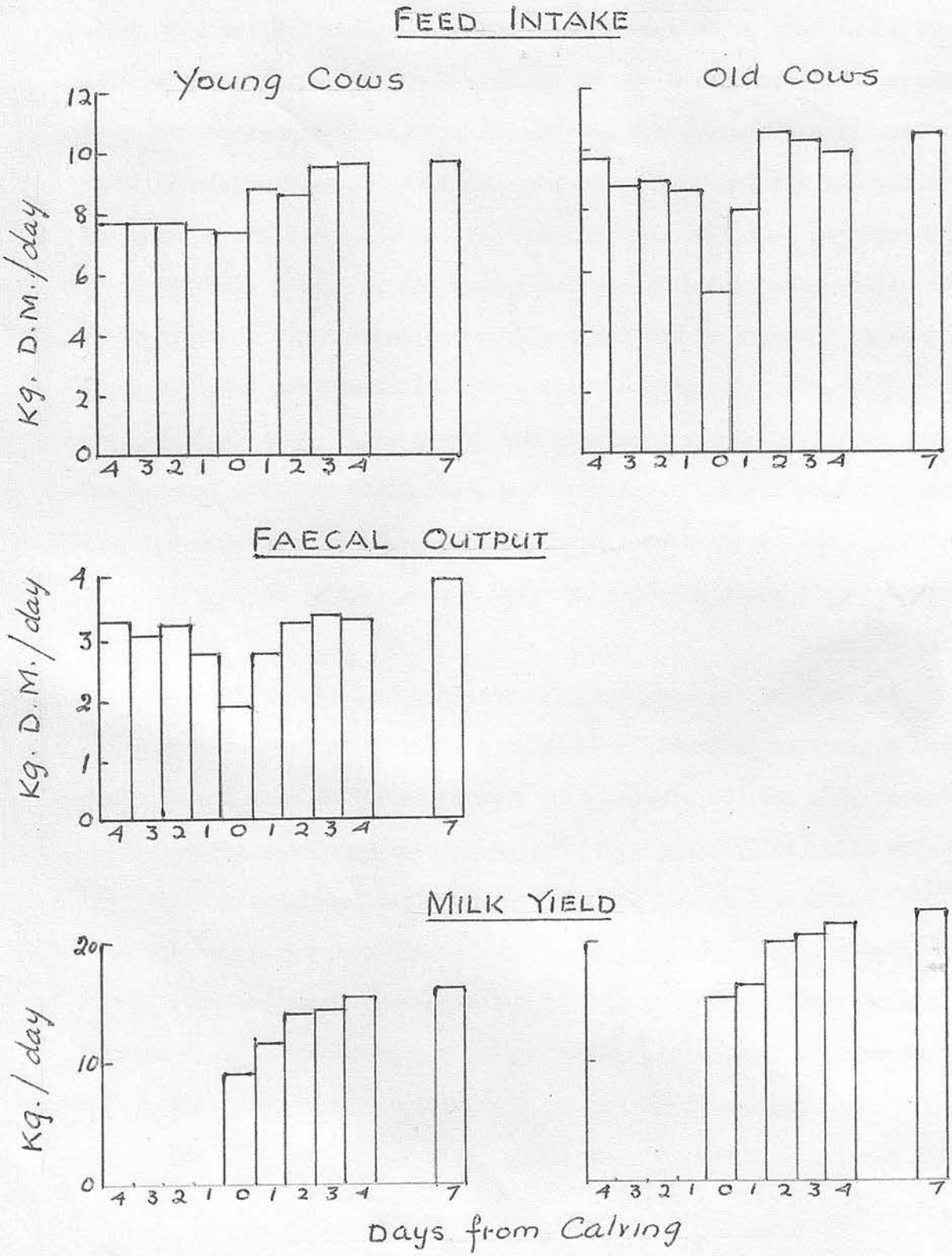
twenty to thirty pounds per day and hay was offered in quantities adjudged sufficient to provide a small excess over each twenty-four hour period. Italian ryegrass hay, which was of less than average quality due to an abnormally bad summer, was used in all but two of the experiments, when Timothy hay was fed. The concentrates were fed before milking time and residues collected one hour later; hay was fed after milking and the residues collected before the next milking; roots were fed in the middle of the morning and the residues collected after about two hours.

Collection of faeces:- Faeces was collected from three of the young cows at frequent intervals during the day. Each day's output was bulked, mixed and an aliquot taken and dried at 100°C to constant weight. Little was washed away by urine, and although some contamination occurred this would not have affected the dry matter faecal output significantly.

Analysis of foodstuffs:- The moisture contents of feeds and residues were determined in duplicate by drying aliquots to constant weight in an oven at 95°C and the samples were then milled. Calcium was determined by the AOAC (1955) macromethod for plants and phosphate by the AOAC (1955) volumetric method for fertilisers after preliminary ashing of the sample in the presence of calcium acetate and the extraction of the ash with dilute nitric acid. Estimations were repeated where the differences between duplicates exceeded two per cent of their mean.

Rumen sounds:- Rumen sounds were estimated by auscultation of the left paralumbar fossa and the sounds evaluated by the following scoring system:-

FIGURE 13 Mean feed intakes, faecal outputs and milk yields of young and old cows before and after calving.



From Tables A1, A2 and A3.

0. No sounds

1. Background sounds similar to those produced by cutaneous twitching.

2. Continuous background sounds occasionally interspersed with sounds like 'peals of thunder' which were usually associated with eructation.

3. Frequent 'thunder' sounds associated with most but not all the rumen contractions.

4. Almost continuous 'thunder' sounds.

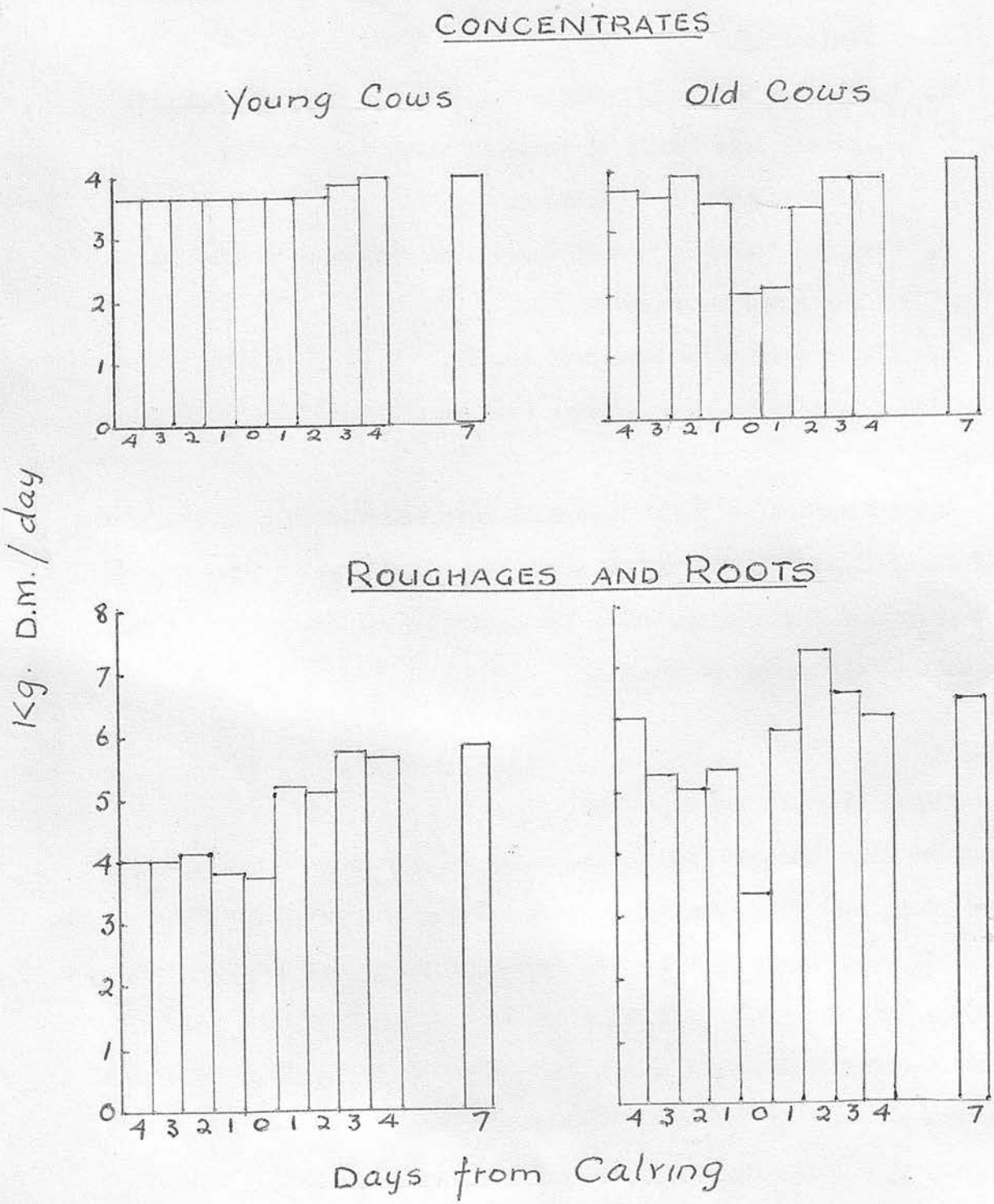
Where doubt existed in classifying a case, an intermediary score was given.

Rumen Movements:- Rumen movements were recorded for periods of ten to fifteen minutes by the method described in Section I. The animals were prevented from eating during the recording periods but no attempt was made to discourage rumination.

Results

Figure 13 shows the mean feed intake and milk yields of the five young and four old cows (cow 4M excluded) and the mean faecal output of three young cows from four days before calving to seven days after calving. The daily feed intake of the young cows remained almost constant until calving, then rose from 7.36 kilograms to over 9.5 kilograms from the third day after calving. In contrast, the food consumption of the old cows was slightly higher initially than for young cows, but dropped steadily by a total of forty-five per cent to a mean food intake of

FIGURE 14 Concentrates and Roughages consumed by young and old cows before and after calving.



From Tables A4 and A5.

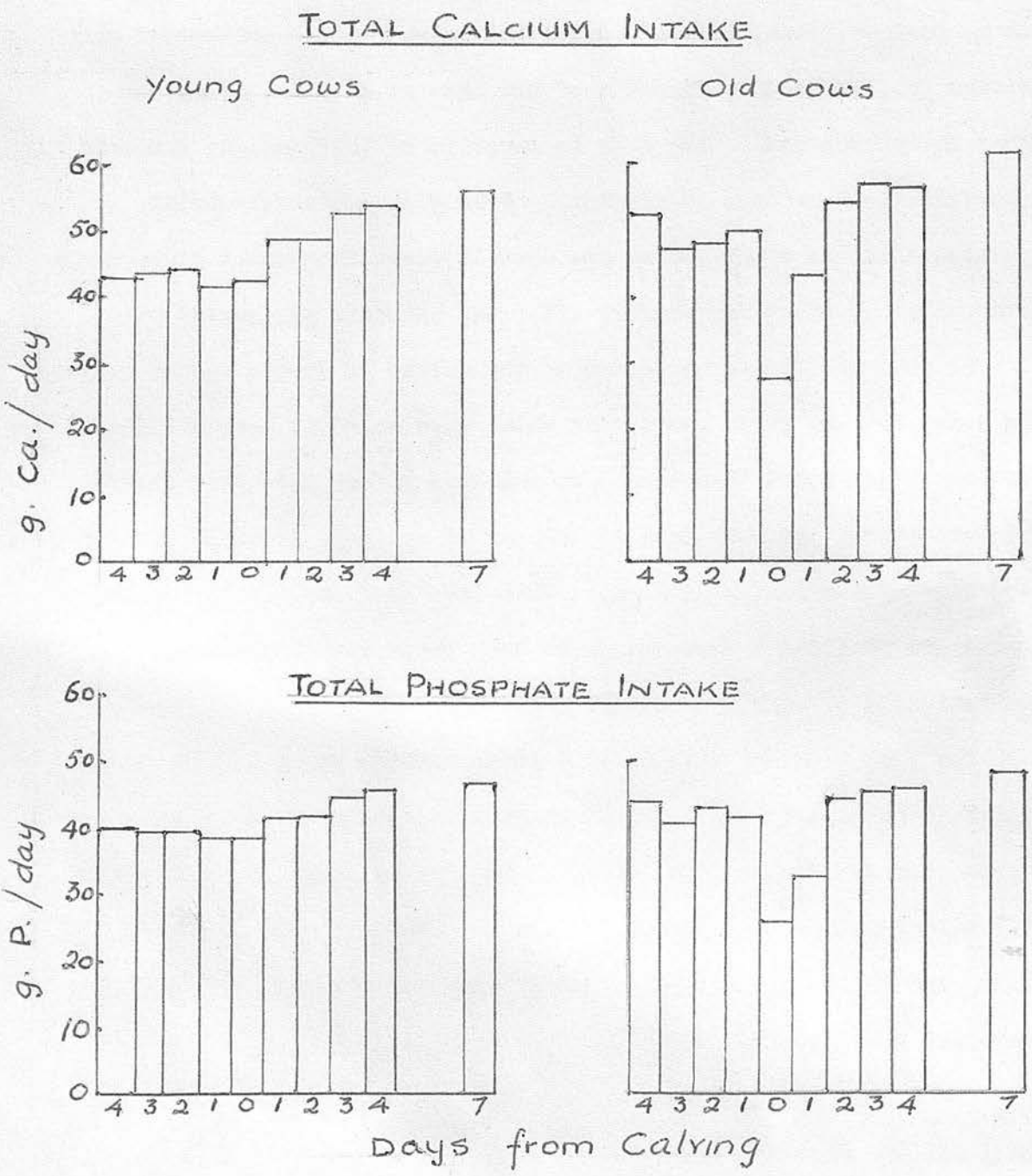
5.3 kilograms on the day of calving. Three of the animals recovered their appetites quickly, but the appetite of the cow which subsequently developed milk fever remained depressed for about one day. There was no real evidence, however, that this animal unduly influenced the average fall in feed consumption of old cows at calving, since the other three cows had a mean loss in appetite of thirty-eight per cent compared with four days precalving. From visual observation it appeared that the appetites of the cows improved from about eight hours after calving, which was shortly after the placenta was passed.

No information was collected on the volume of faeces passed by the old cows, but the three young cows which were examined passed fifteen per cent less faeces than normal on the days before and after calving, and forty-three per cent less on the day of calving, in spite of their food consumption remaining high. This loss in faecal yield was so marked and consistent that even with only three cows the results are statistically of very high significance.

The cows were not milked until after calving so no information is available on colostral secretion before parturition. The young cows calved with an average yield of 9.2 kilograms per day, compared with 15.3 kilograms per day for the old cows. Production increased rapidly during the following week in the young cows but only two of the old cows increased their yields.

The loss of appetite among the older cows was evenly distributed among all the dietary components (Figure 14), although individual animals showed preferences for different foods. In all animals the increase in

FIGURE 15 Total calcium and phosphate intakes of young and old cows before and after calving.



From Tables A6, A7 and A8.

FIGURE 16 Relation between feed intake on the day of calving as a percentage of the four-day precalving value and number of calving.

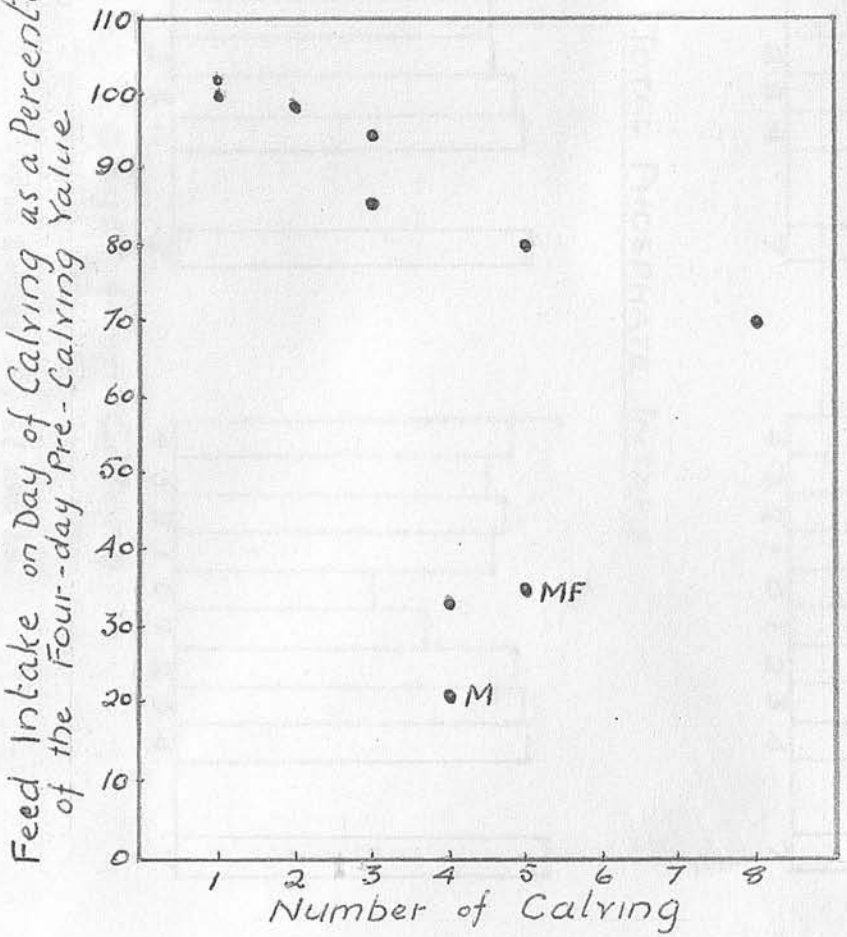
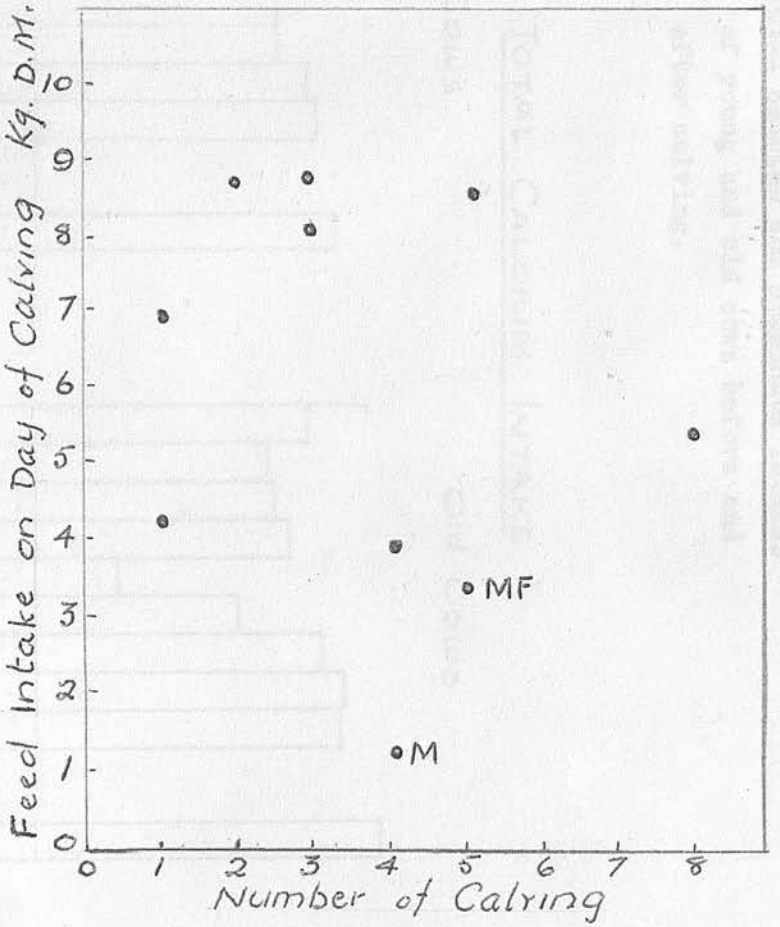


FIGURE 17 Relation between feed intake on day of calving and number of calving.



M — Clinical Metritis Two Days after Calving
MF — Milk Fever Sixteen Hours after Calving.

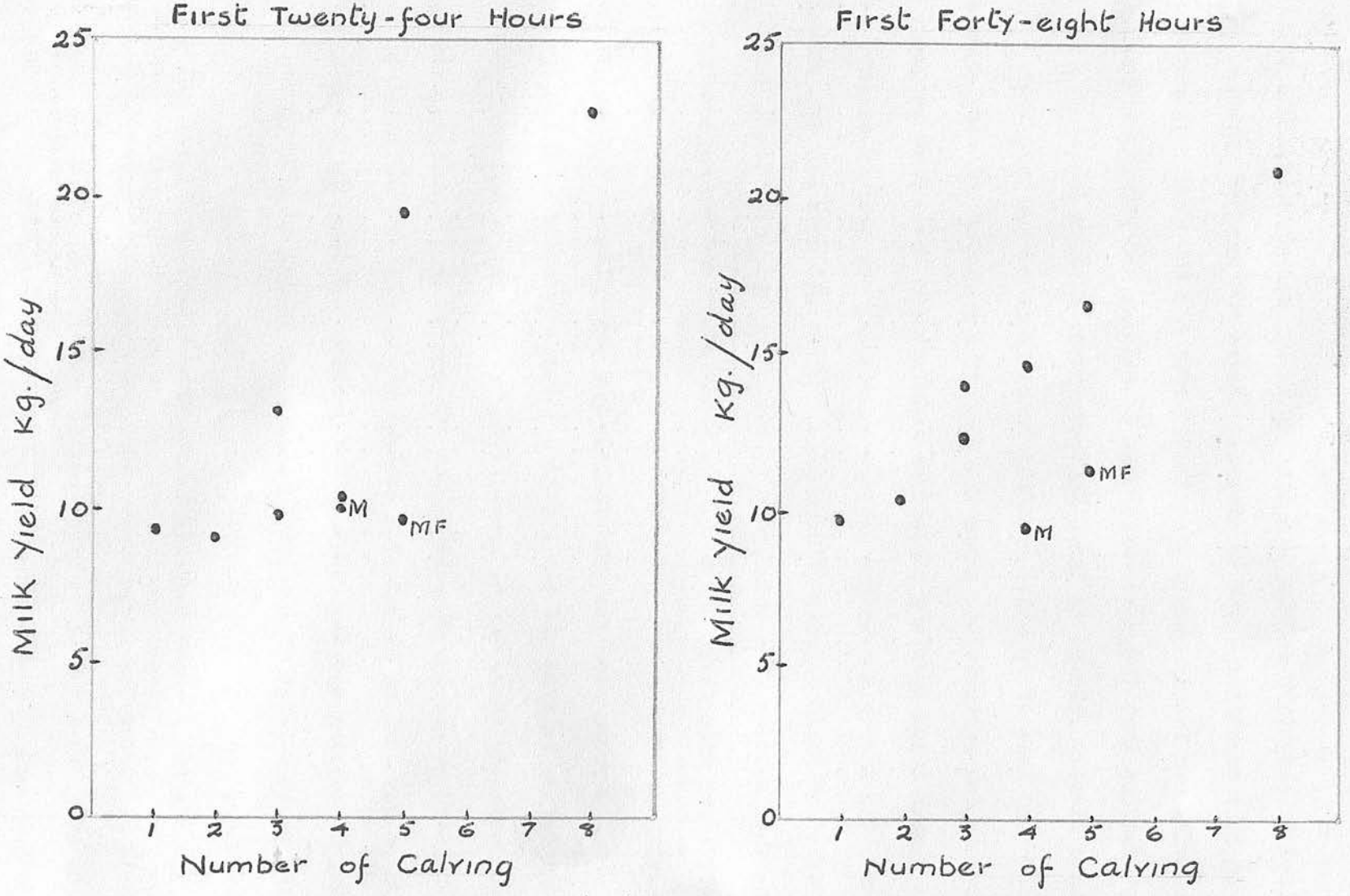
feed intake after calving was largely in hay consumption, this being the only constituent fed to excess. Since the loss of appetite at calving was fairly evenly distributed over all the food components, it follows that the mean variations in calcium and phosphate intakes were closely related to those in feed intake (Figure 15).

The loss of appetite possibly became more severe with each succeeding parturition, since the data from seven of the cows showed a linear trend when the percentage reduction in their food consumption from the fourth day before calving to calving was plotted against the number of their calving (Figure 16). Three old cows did not adhere to this pattern, but this group included the animals which later developed milk fever and metritis. The other cow was apparently in normal health.

The young cows ate between four and ten kilograms dry matter per day before calving and the old cows between five and twelve kilograms; consequently there was no correlation between the actual weight of food consumed on the day of calving and the number of the calving (Figure 17). There was also no correlation between the amount of food eaten on the day of calving and the initial milk yields, although the three cows with the greatest loss of appetite produced low yields of milk on the day of calving.

Figure 18 shows the relation between number of calving and total milk yield for the first twenty-four and forty-eight hours of lactation. The older cows tended to have greater yields of milk and the trend was more consistent when the milk yields were averaged over the first two

FIGURE 18 The relation between number of calving and the milk yield of cows during the first twenty-four and forty-eight hours of lactation.



From Table A3.

FIGURE 19 Relation between mean daily milk yield of thirty-five cows during the first forty-eight hours of lactation and number of calving.

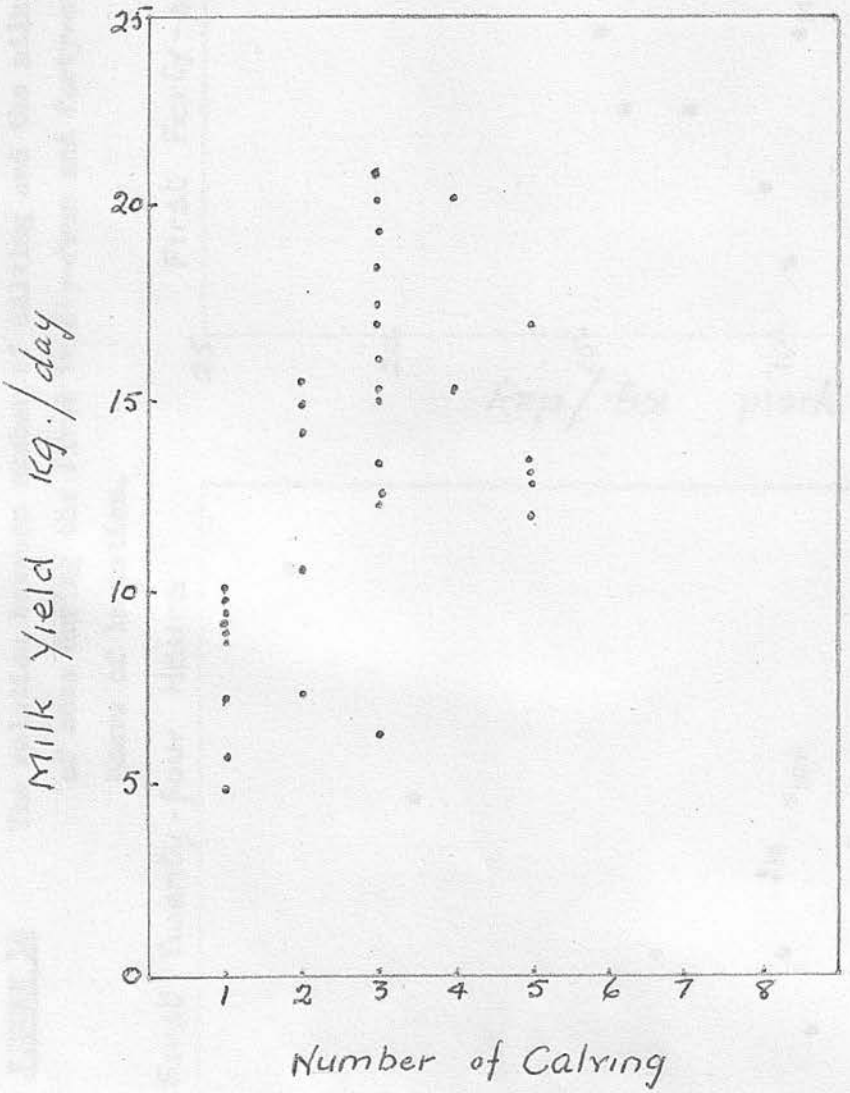
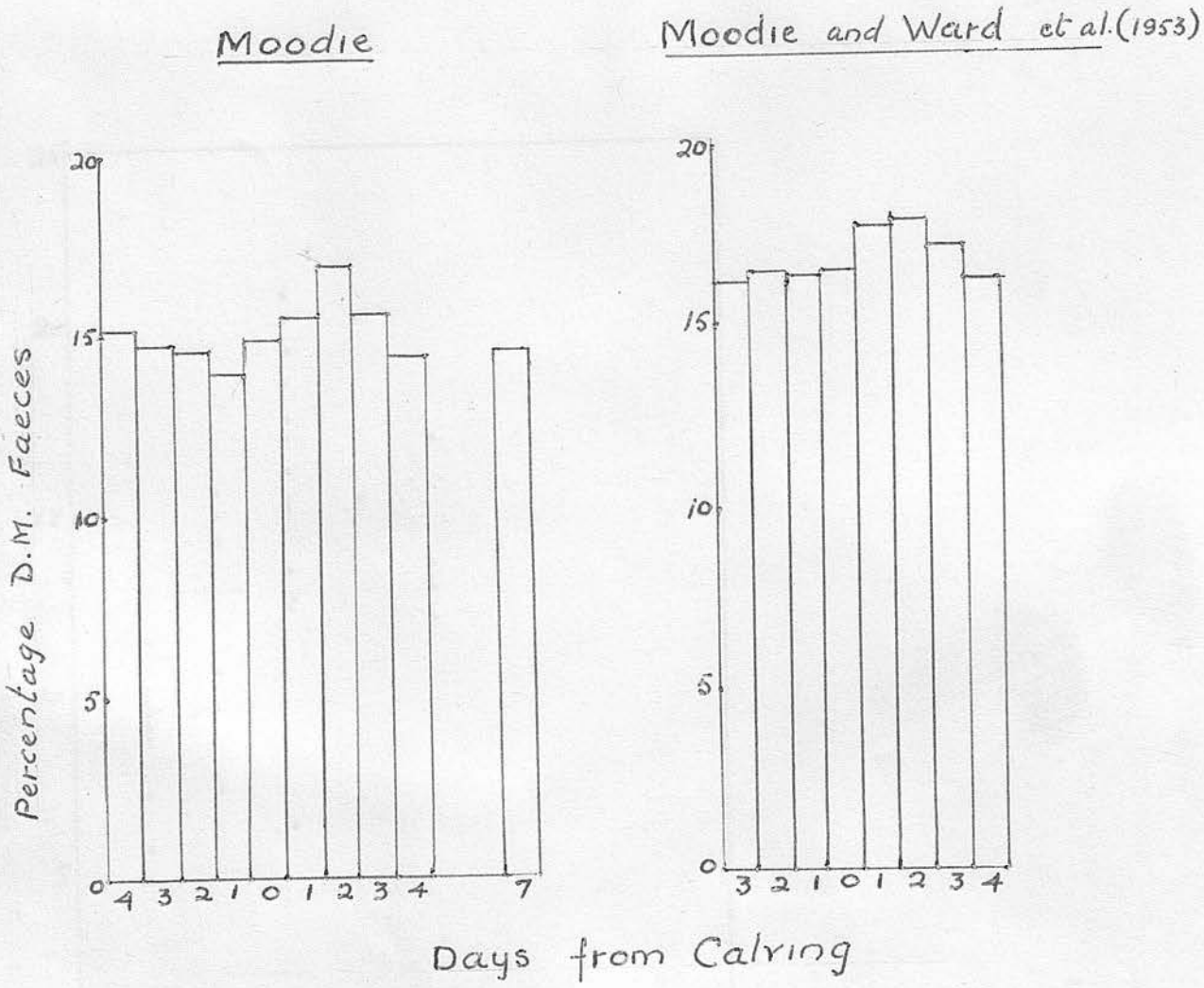


FIGURE 20 Percentage dry matter in faeces of cows before and after calving.



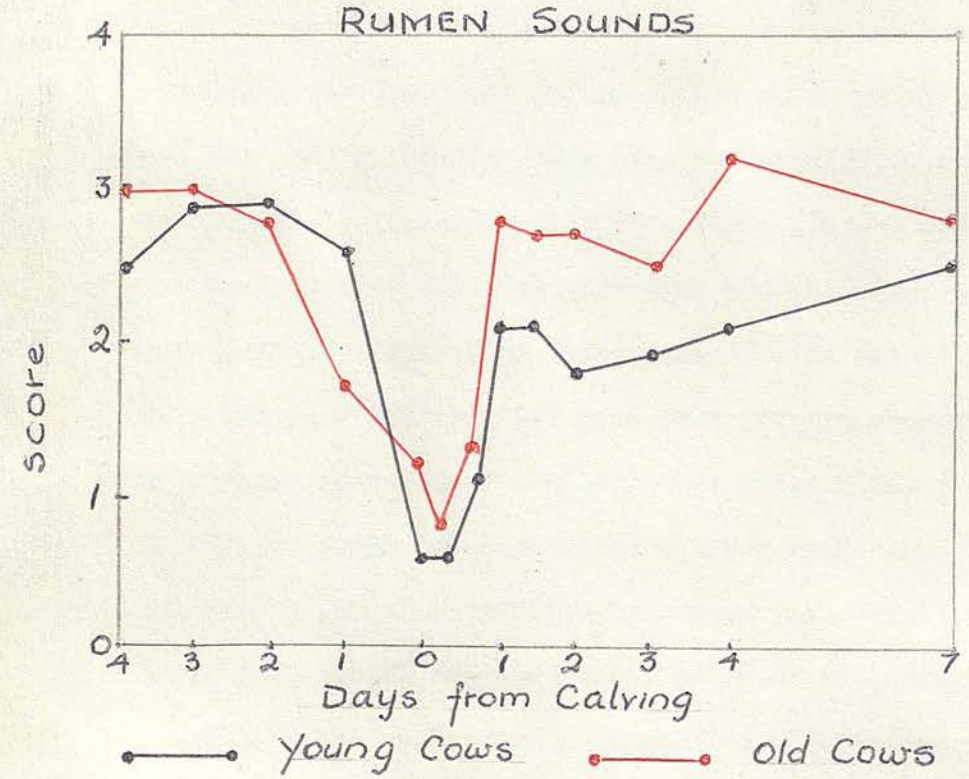
From Tables A9 and A10.

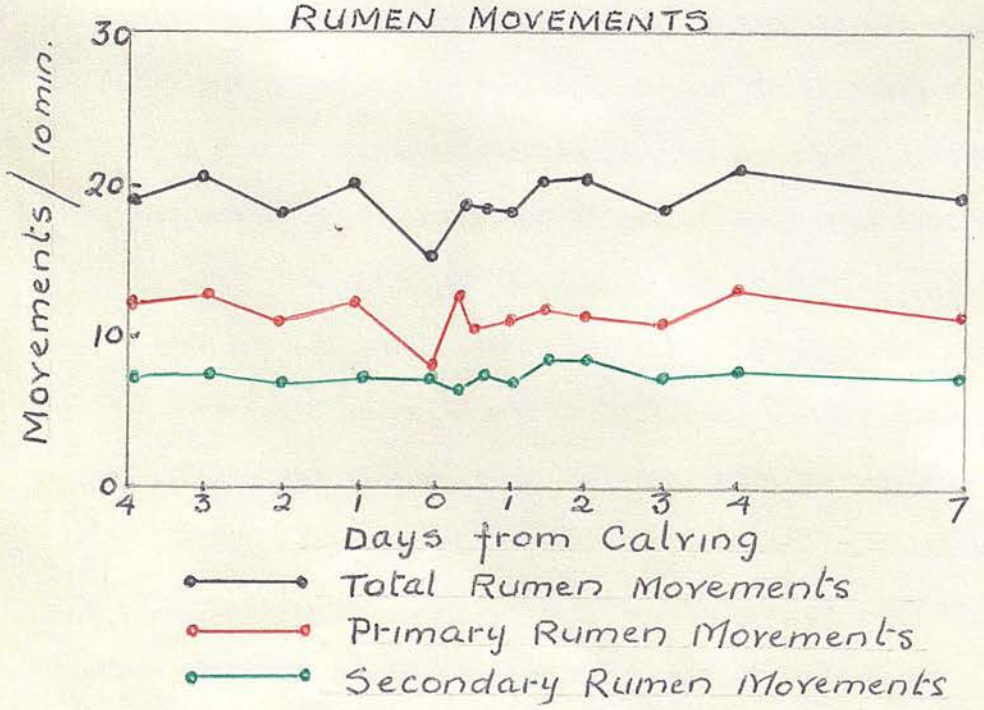
days of lactation. The metritis and milk fever cases yielded less milk than the trend would indicate was normal.

Figure 19 shows the milk yields during the first forty-eight hours of lactation of thirty-five cows which calved in the same herd over a twelve month period. All cows with a history of illness at calving have been excluded from this figure. The data in this figure, however, is not so reliable as in the previous figure, since some of the older cows may not have been fully milked out, while on occasion a small milking taken immediately after calving may not have been recorded. Both these factors would tend to reduce the yield recorded over the first forty-eight hours after calving. Despite this failing, however, it is quite clear from the figure that cows at their third or subsequent calving gave more milk than heifers.

The reduction in food consumption and in faecal output of cows suggests that there may be some reduction in alimentary activity at calving. Further evidence of this is obtained by examining the moisture content of the faeces which was passed, since alimentary stasis is clinically associated with some degree of constipation. Figure 20 shows the dry matter content of the faeces of the three cows from which samples were obtained and while the method of collecting the faeces did not preclude the possibility of its dilution with urine or water, there is evidence of some increase in dry matter content in the days following calving. The changes were not significant, but by combining the data with the results obtained by Ward et al. (1953a) for two Jersey cows, two Jersey heifers and four Holstein or Guernsey cows,

FIGURE 21 Rumen sounds scores for seven cows and rumen movements of five cows before and after calving.





From Tables A11-A14.

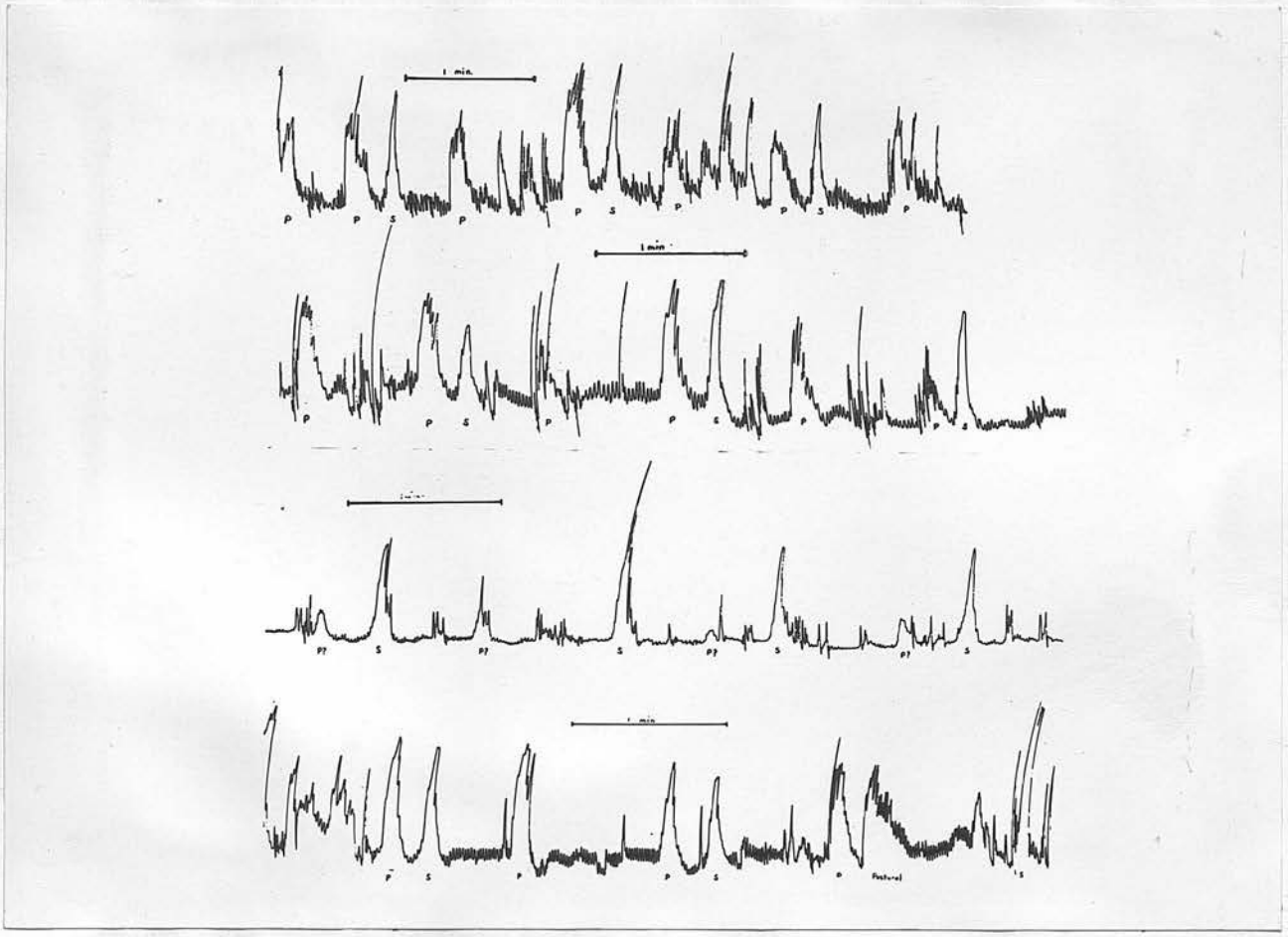
the trend can be confirmed and significant differences between days are found.

Rumen activity was also recorded and the results are shown in Figure 21. The rumen sounds scores, relating to four young and three old cows, dropped markedly from prepartum mean values of 2.5 and 3.0 to under 1.0 shortly after parturition. Recovery to values above 2.0, which may be regarded as fairly normal, took about one day, but maximum scores were not recorded until later. There were no apparent differences between young and old cows, the rumen sounds always disappearing at the time of calving and in some animals even the sound of eructation was inaudible on auscultation at the paralumbar fossa.

Total rumen movements showed a slight reduction in frequency at the time of calving which can be attributed entirely to changes in the primary movements. These results were obtained from three young and two old cows.

Much more noticeable, however, was the evidence of weakening of the primary waves compared with the secondary movements in three of the five animals examined. Typical findings are illustrated in Figures 22-24 which show tracings from cows at their first, second and fifth calvings respectively. Figure 22 shows a very marked reduction in ruminal activity at calving, both in the frequency of the primary waves and their strength compared with the secondary waves. This effect was not quite so marked in the cow recorded at her fifth calving (Figure 24) and no change could be detected at any time in the cow at her second

FIGURE 22 Rumen movements recorded from the left paralumbar fossa of a cow at her first calving (Cow 1b).



A - Normal tracing. Movements 18/10 min. Sounds score 3.0. Heifer very restless.

B - Seven hours pre-calving. Movements 18/10 min. Sounds score 0. Primary waves weakening.

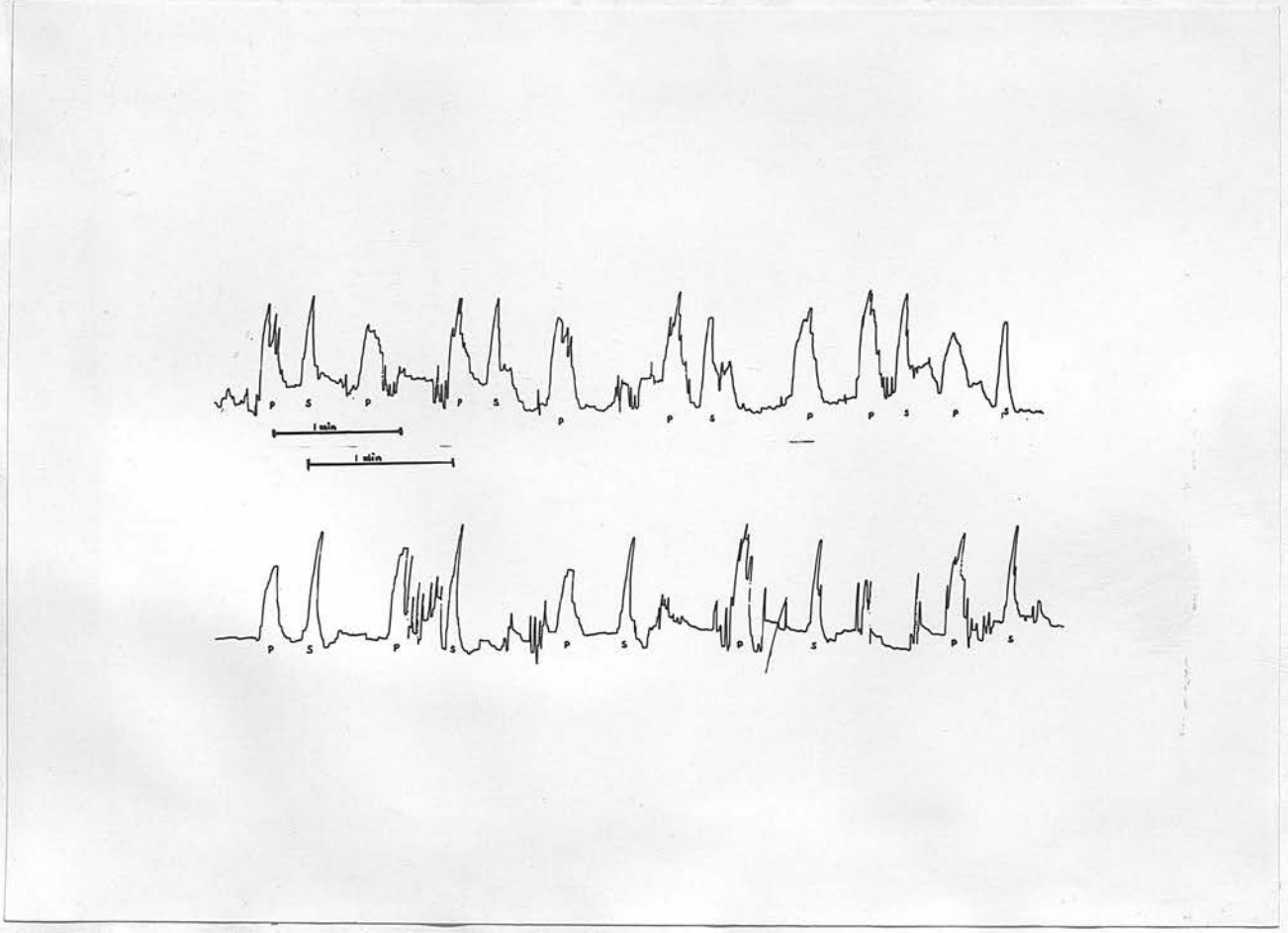
C - Calving. Movements 9/10 min. Sounds score 1.0. Primary waves very weak.

D - Eight hours after calving. Movements 14/10 min. Sounds score 1.0.

P - Primary waves

S - Secondary waves

FIGURE 23 Rumen movements recorded from the left paralumbar fossa of a cow at her second calving (Cow 2).



A - Normal tracing. Movements 22/10 min.
Sounds score 3.0.

B - Half hour after calving. Movements 17/10 min.
Sounds score 0.

P - Primary waves

S - Secondary waves

FIGURE 24 Rumen movements recorded from the left paralumbar fossa of a cow at her fifth calving (Cow 5).

A - Normal tracing. Movements 17/10 min. Sounds score 3.0.

B - Twenty hours pre-calving. Movements 18/10 min. Sounds score 1.5. Primary waves reduced in height.

C - Half hour after calving. Movements 12/10 min. Sounds score 1.5. Primary waves small.

D - Eight hours after calving. Movements 16/10 min. Sounds score 2.0.

P - Primary waves

S - Secondary waves

calving (Figure 23).

Discussion

There is good evidence that the incidence of milk fever in cows increases with advancing age (Jonsson 1960b) and that the serum calcium levels of old cows at calving are lower than those of young cows (Moodie, Marr and Robertson, 1955). These observations can hardly be explained simply as reductions in the general ability of old cows to mobilise calcium from the bone or the digestive tract, since hypocalcaemia develops most frequently at the time of calving and not at the peak of lactation, when the efficiency of mobilisation would be most sorely tested. Thus we must look for factors peculiar to the newly calved cow which may influence the calcium balance and which are more pronounced in old cows. As already discussed, the most important factors would have to do with milk secretion, mobilisation from the somatic tissues and mobilisation from the alimentary canal.

In this section evidence has been produced of a reduction at calving in the feed consumption of the older cows, which also tended to calve with higher colostral secretions over the first twenty-four and forty-eight hours of their lactations. The milk yields on the first day averaged 9.2 kilograms and 15.3 kilograms for young and old cows and at this time calcium consumption was 42.2 grams and 27.3 grams respectively. On the second day the corresponding values were 11.6 kilograms and 16.4 kilograms of milk and 48.4 grams and 43.0 grams of calcium. These two factors operating together could possibly increase the dependence of old cows on the skeleton as a source of minerals,

but there is some evidence of a reduction in the mobility of bone calcium in such animals (Hansard et al., 1954; Conrad et al., 1956), so the net result could be disequilibrium of the serum calcium.

The possible role of milk yield and food intake in precipitating hypocalcaemia will be discussed more fully in the following sections and it is only necessary at this stage to consider the factors which might influence milk yield and food intake soon after calving. The heavier milk yield of older cows during the first two days of lactation seems to be a general feature, as has now been confirmed by Payne (1963b). The initial yield is possibly influenced by factors other than age, but of this there is little evidence in these experiments. For example, the level of feeding before calving might be a factor, but the amounts of concentrates consumed by the young and old cows were almost identical, although the calcium and phosphate intakes of the old cows were about ten per cent higher than young cows due to a higher consumption of roughages and succulent foods. Culling in the herd might also influence the average initial milk yields of older cows, but again there is no supporting evidence. In this herd, as in the national herd (Leech, Davis, Macrae and Withers, 1960), most animals are culled for infertility or disease conditions such as mastitis and there is no reason to believe that such conditions would arise more frequently in cows with low initial milk yields. Moreover, the values obtained from cows of each lactation group (Figure 19) have roughly the same distributions, which suggest that the culling was having little effect on initial milk yield. So it would seem reasonable to suppose that

the higher initial yields of the older cows, at least up to the third calving, are a normal physiological mechanism in the same way that the heaviest lactational yields are normally expected at the third or fourth lactation, and indeed, a wider survey might show the two factors to be correlated.

The changes in dietary intake in these trials are largely reflected by the dietary calcium and phosphate data. The feed intake of the young cows dropped by about four per cent on the day of calving and of the old cows by forty-five per cent. In the single milk fever case, calcium intake fell from over sixty grams daily on the fourth, third and second days before calving to twenty grams on the day of calving and there was little evidence of any increase in the calcium intake on the first day after calving. These results are in keeping with the observations of Ward, Blosser and Adams (1952), who attempted to carry out balance trials on Jersey cattle. They found that in heifers the calcium intake dropped only slightly, but in mature cows the intake fell from about fifty-five grams per day five to three days before calving to about thirty grams on the day of calving, a reduction of some forty-five per cent, and in three mature cows which developed milk fever the calcium intake dropped even further.

The type of food chosen by cows at calving did not appear to be confined to any one group, the consumption of both concentrates and roughages by older cows being about halved, and probably depended on the time of day that the refusals occurred in relation to the feeding regime. The loss of appetite cannot be related to change in palatability of the

diet, since the same food was offered throughout each experiment; nor can it be associated with mineral or trace element deficiency, since the appetite returned to normal as soon as the calving was complete, and except for cow number eight there was no evidence of overeating on the day prior to calving which would suggest that appetite had been over-satisfied.

There was, however, evidence of reduced motility of the alimentary canal at the time of calving. The faecal output was reduced in all three young cows in my own experiments and in three out of four of those of Ward et al., (1953a) and the dry matter content of the faeces increased shortly after calving. There was also evidence of a reduction in the frequency of the primary rumen movements and a loss of rumen sounds about the time of calving, and of weakening of the primary rumen contractions in some animals before calving. The cause of the reduced activity of the alimentary canal will be discussed more fully later, but was not likely to be the result of hypocalcaemia since in four of the cows where there was a marked reduction in the dietary intake, the change was noticeable some twenty-four hours before serum calcium concentrations fell below nine milligrams per cent. Payne (1964) observed that cows ate and chewed cud normally during EDTA infusion experiments, provided the serum calcium concentrations did not change too rapidly and remained above six milligrams per cent.

The digestive upsets at the time of calving suggest that calcium and phosphate absorption could be impaired by a failure in the movement

of nutrients to sites favourable for absorption, but definite proof of this must await further study. The actual reduction in nutrients consumed is not likely to be important in the development of hypocalcaemia and hypophosphataemia unless it persists for more than one or two days. Large reserves of calcium and phosphate occur in the rumen and even with complete starvation of heavily milking cows there is a three to four day time lag before serious hypocalcaemia develops (Robertson, Paver, Barden and Marr, 1960). Reduction of nutrient intake could, perhaps, be of some importance in the later development of hypocalcaemia since in the third and fourth days after calving the rumen reserves might be diminished. At this time the dietary intake of calcium by the old cows was no higher than for the young cows, yet their requirement for calcium for milk secretion would be some thirty or forty per cent higher.

The loss of rumen sounds as parturition became imminent was a consistent feature of all cows examined but cannot be explained by complete inactivity of the rumen, since the movements recorded were quite normal in some cows and only partly inhibited in others, and in no case were the secondary movements, which are predominantly associated with eructation (Stevens and Sellers, 1959), affected. It is difficult to offer an adequate explanation for this discrepancy between the rumen movements and sounds but it could be associated with changes in the 'degree of fill' of the rumen.

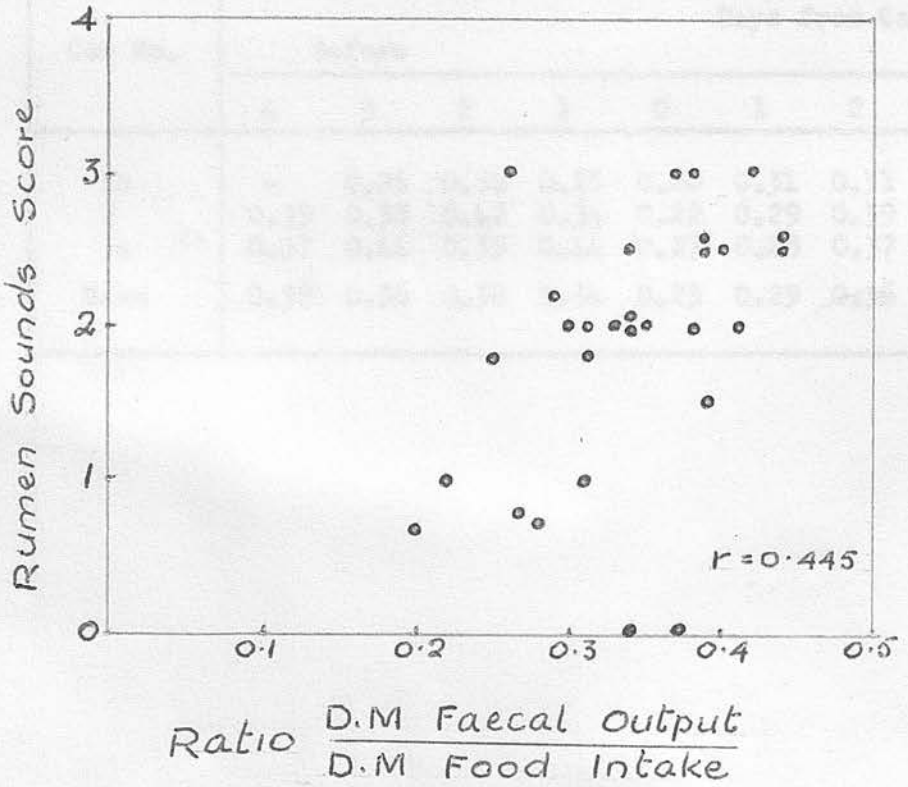
A crude estimate of changes in the total solids contained within the alimentary canal can be obtained by comparing the daily faecal dry

TABLE VI

Ratio between dry matter excreted in the faeces and the Dry Matter of the Food Consumed During 24 Hrs. $\frac{\text{(D.M. Faeces)}}{\text{(D.M. Food)}}$ of three cows before and after calving.

Cow No.	Days from Calving									
	Before					After				
	4	3	2	1	0	1	2	3	4	7
1b	-	0.26	0.34	0.25	0.20	0.31	0.31	0.33	0.30	0.35
2	0.39	0.38	0.42	0.34	0.22	0.29	0.39	0.34	0.40	0.38
3a	0.37	0.44	0.39	0.44	0.27	0.28	0.37	0.34	0.31	0.41
Mean	0.38	0.36	0.38	0.34	0.23	0.29	0.36	0.34	0.34	0.38

FIGURE 25 Correlation between rumen sounds score and the ratio of the dry matter faecal output to dry matter feed intake.



From Tables VI and All.

matter output with the dry matter food intake. Table VI shows that the ratio of faecal output to food intake dropped by about forty per cent on the day of parturition in three normal calving cows and it is unlikely that a change of this magnitude would be due solely to an increase in the digestibility of the food. The values of these ratios are positively correlated with the rumen sounds score ($r = 0.455$; $n = 29$; $P = 0.02$) thus supporting the possibility that in certain circumstances the rumen sounds score is influenced by the 'degree of fill' of the rumen (Figure 25). These observations suggest that faecal output may be a better twenty-four hour indicator of intestinal activity than food intake.

The apparent discrepancy between the recordings of rumen movements, where the most severe dysfunction was observed in a heifer, and the feed intake data, where the older animals were more seriously affected, is simply explained; the former measured activity over short periods of about fifteen minutes while the latter gave an average daily picture. In the heifer the rumen movements seven hours before calving and eight hours after calving (Figure 22) were substantially normal although there was a marked diminution in rumen activity at calving, so the animal would have had plenty of time to eat her ration within the twenty-four hour period for recording food intake. Transient alimentary inactivity of this nature may not upset the serum calcium concentration appreciably and both severity and duration of the gut stasis must be measured. This is possibly done most simply by observing either daily food intake or faecal output. The faecal output data in this paper and in the

literature is too sparse to permit comparisons between calving animals of different ages but there is good evidence that appetite is more seriously affected in older cows.

Summary

The daily food intake, calcium and phosphate intakes and milk yield of ten cows at calving are reported, along with faecal output data for three cows. The activity of the rumen was assessed on six cows by a rumen sounds scoring system and the frequency and the amplitude of the movements of the dorsal sac were recorded on five cows.

The results show that the old cows ate less food and produced more milk than the young cows during the first two days after calving. The changes in faecal output and rumen activity at calving suggest that the loss of appetite resulted from alimentary tract stasis. It is not known whether this stasis was more severe in old cows than in young cows, but there is evidence that it was not the result of hypocalcaemia.

SECTION III

THE RELATIONSHIP BETWEEN THE BIOCHEMICAL CHANGES, AND BETWEEN THE BIOCHEMICAL AND PHYSIOLOGICAL CHANGES, IN CALVING COWS

In Section II observations were made on some physiological changes in cows at calving which might influence the calcium and phosphate balance. The primary object of this section is to see if some of those changes, namely, feed, calcium and phosphate intakes and milk yield, are correlated in any way with the serum calcium and blood phosphate levels. At the same time, blood collected from some of the cows was analysed for glucose, pyruvic acid, lactic acid and citric acid content, to test the suggestions advanced by Ward, Blosser, Adams and Crilly (1953) that there may be some impairment of Kreb's cycle, and to study further the observation of Blosser and Smith (1950) that serum calcium and citric acid levels show similar changes in cows at calving.

Materials and Methods

Animals:- The observations were made on the ten cows used in the previous section.

Sampling:- Blood samples were withdrawn from either the mammary or jugular veins, the mammary vein being used whenever it was sufficiently developed for serial sampling. One clotted and one heparinised sample were collected on each occasion. In experiments involving lactic or pyruvic acid estimations, a portion of the sample was

immediately precipitated in freshly prepared ice-cold ten per cent w/v trichloroacetic acid. This preparation was kept chilled until all estimations were completed and was used for the determination of inorganic phosphate and citric, lactic and pyruvic acid concentrations in whole blood. Samples required for glucose estimation were collected in heparinised bottles containing 0.3 mg. sodium iodoacetate per millilitre of blood.

Estimations:- Serum calcium was estimated in serial batches of samples by the Clark and Collip (1925) modification of the Kramer-Tisdall method, frequently one or two samples being re-analysed with the next batch to check for batch differences. Inorganic phosphate estimations on whole blood were completed in all cases within eight hours of sampling, using the method of Fiske and Subbarow (1925). Blood glucose was determined by the method of Somogyi (1952) within a few hours of collection, and citric, lactic and pyruvic acids by the techniques of McArdle (1955), Barker and Summerson (1941) and Friedmann and Haugen (1943) respectively. All estimations were carried out in duplicate and serum calcium and blood inorganic phosphate determinations were repeated if the differences between the duplicates exceeded 0.3 mg. per cent and 0.2 mg. per cent respectively. Serum calcium and blood inorganic phosphate was estimated in samples from all the cows, citric acid in eight cows, glucose and pyruvic acid in five cows and lactic acid in four cows.

Statistical analyses:- Statistical analyses of the results were based on the recommendations of Snedecor (1946), but the possibility of

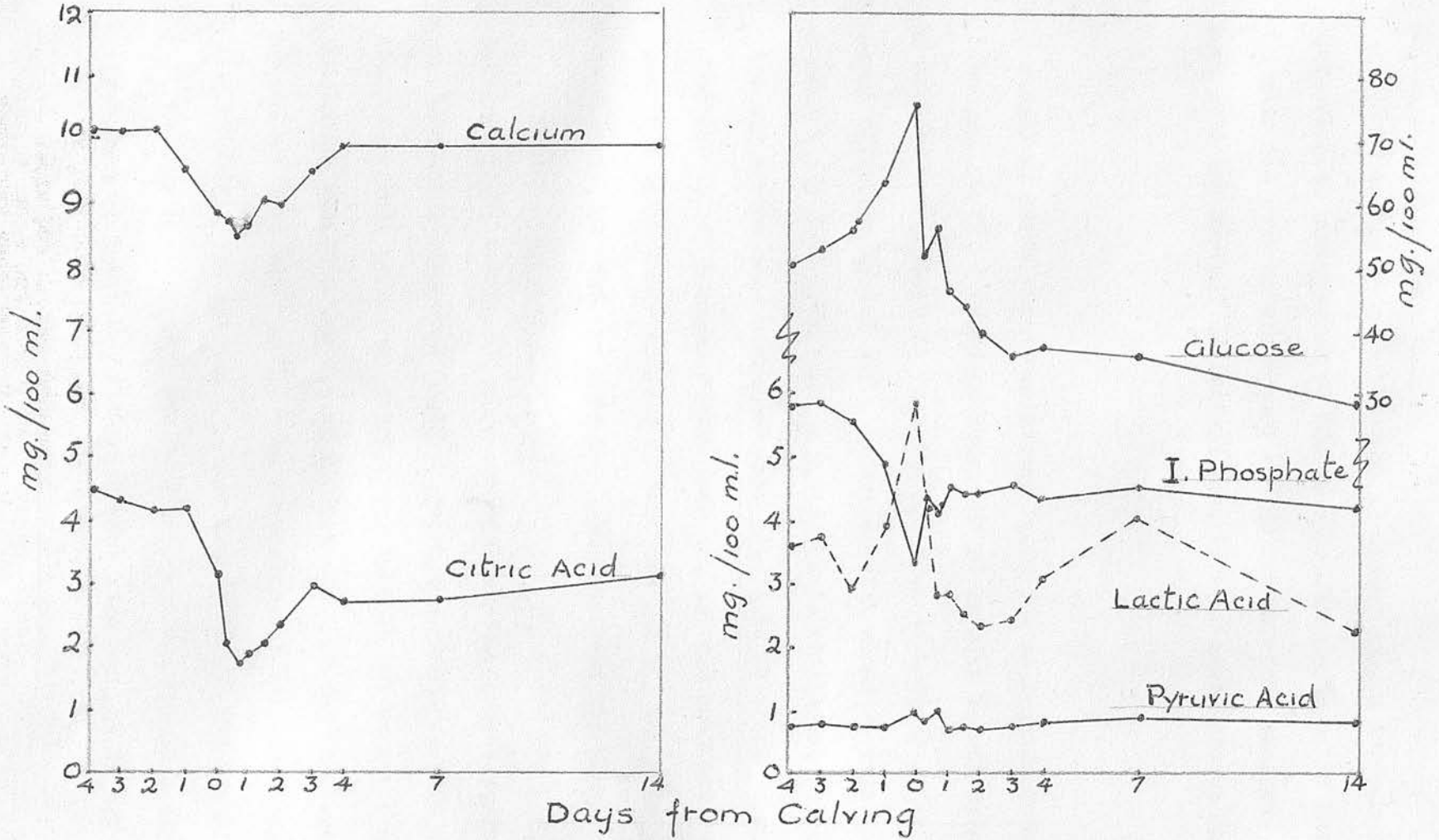
serial correlation between readings on successive occasions cannot be excluded and tests of significance should be interpreted accordingly. As in the previous section, cow 4M has been excluded from all mean values, but correlations used to test mechanisms include the data from this cow.

Results

The mean changes in the blood constituents are shown in Figure 26. The concentration of calcium in the serum averaged 10.09 mg. per cent before calving, dropped to 8.85 mg. per cent at calving and to 8.46 mg. per cent at sixteen hours after calving. The levels gradually increased thereafter and stabilised at about 9.9 mg. per cent from the fourth day post-partum. Whole blood citric acid concentrations showed the same trends, falling from 4.5 mg. per cent before calving to 3.2 mg. per cent at calving and 1.7 mg. per cent at sixteen hours after calving. The levels gradually increased over the next two days and stabilised at about 2.7 mg. per cent.

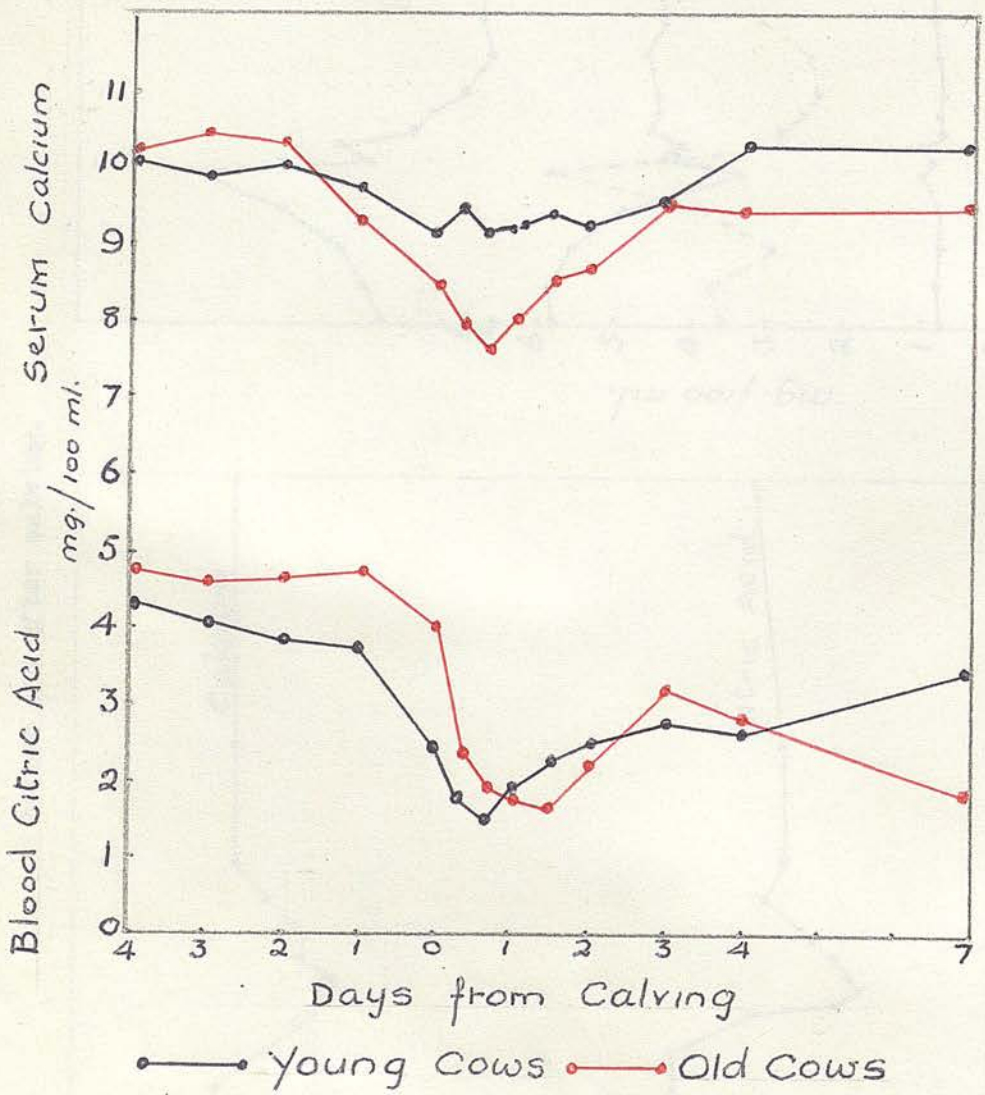
The other constituents which were examined showed their maximum changes in concentration at calving. Blood inorganic phosphate concentrations started to decrease two days before calving, dropping slowly at first from 5.8 mg. per cent and then rapidly to 3.3 mg. per cent at calving. Within eight hours the level rose to over 4.0 mg. per cent and stayed there until the end of the observations. Average whole blood glucose concentrations formed a mirror image of the phosphate changes, rising steadily from 50 mg. per cent four days before calving to a mean of 75 mg.

FIGURE 26 Serum calcium and blood glucose, inorganic phosphate, citric, lactic and pyruvic acid levels of cows before and after calving.



From Tables A15-A20.

FIGURE 27 Concentration of calcium in the serum and citric acid in the blood of young and old cows before and after calving.



From Tables A15 and A16.

per cent at calving. Eight hours later the glucose level had dropped by 20 mg. per cent and from two days after calving mean values of under 40 mg. per cent were obtained.

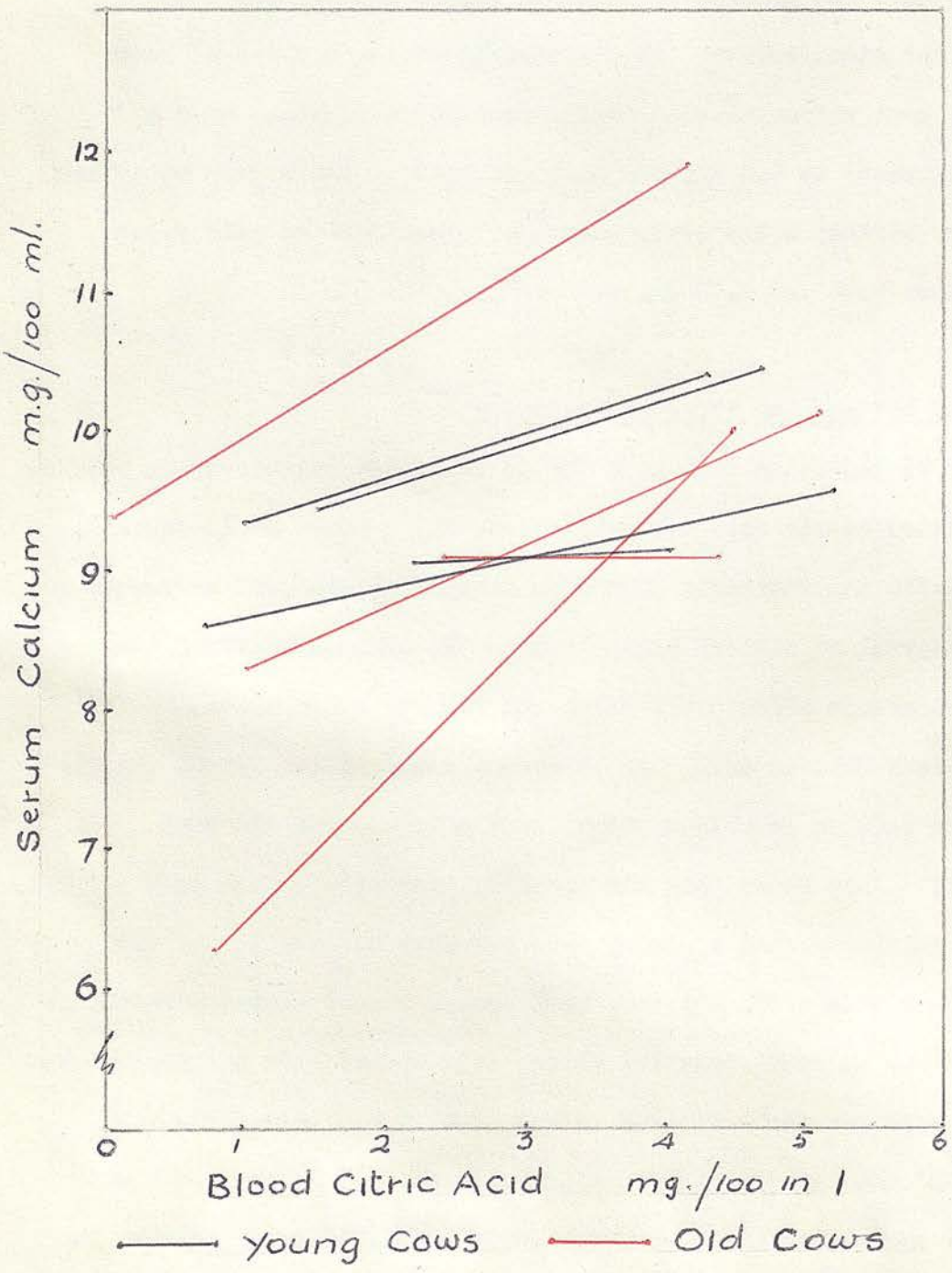
Lactic acid and pyruvic acid values showed only slight changes which were not significant. The initial lactic acid level of under 4.0 mg. per cent was maintained until right up to calving, when a transient increase to 5.8 mg. per cent occurred. The values were back to normal by sixteen hours after calving. Mean pyruvic acid values varied between 0.74 and 1.00 mg. per cent.

Correlations between the blood constituents

Figure 26 indicates that only the correlations between serum calcium and whole blood citric acid concentrations, and between whole blood glucose, lactic and inorganic phosphate concentrations need be considered. The time interval of sixteen hours between the maximum observed changes of these two groups effectively rules out further comparisons.

Sufficient data is available to permit a comparison of the changes in the serum calcium and blood citric acid of young and old cows (Figure 27). This shows that the serum calcium values fell only slightly during the calving period in young cows but more heavily in the old cows. With citric acid, however, both groups showed almost identical changes. Thus in young cows low citric acid values were not necessarily associated with low serum calcium values, but in old cows these two components of the blood altered similarly. An analysis of covariance (Table A21) shows that the regression coefficients of serum calcium on

FIGURE 28 The regressions of serum calcium on blood citric acid for four young and four old cows.



From Table A21.

FIGURE 29 Correlation between the concentration of glucose and lactic acid in the blood of cows before and after calving.

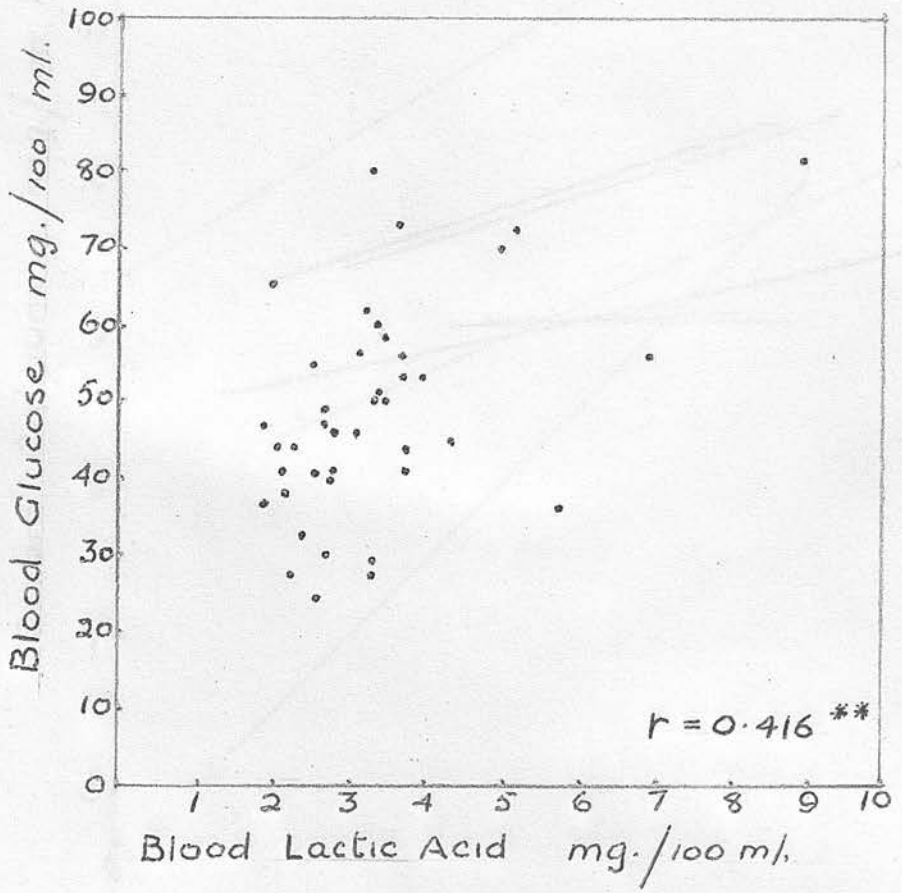
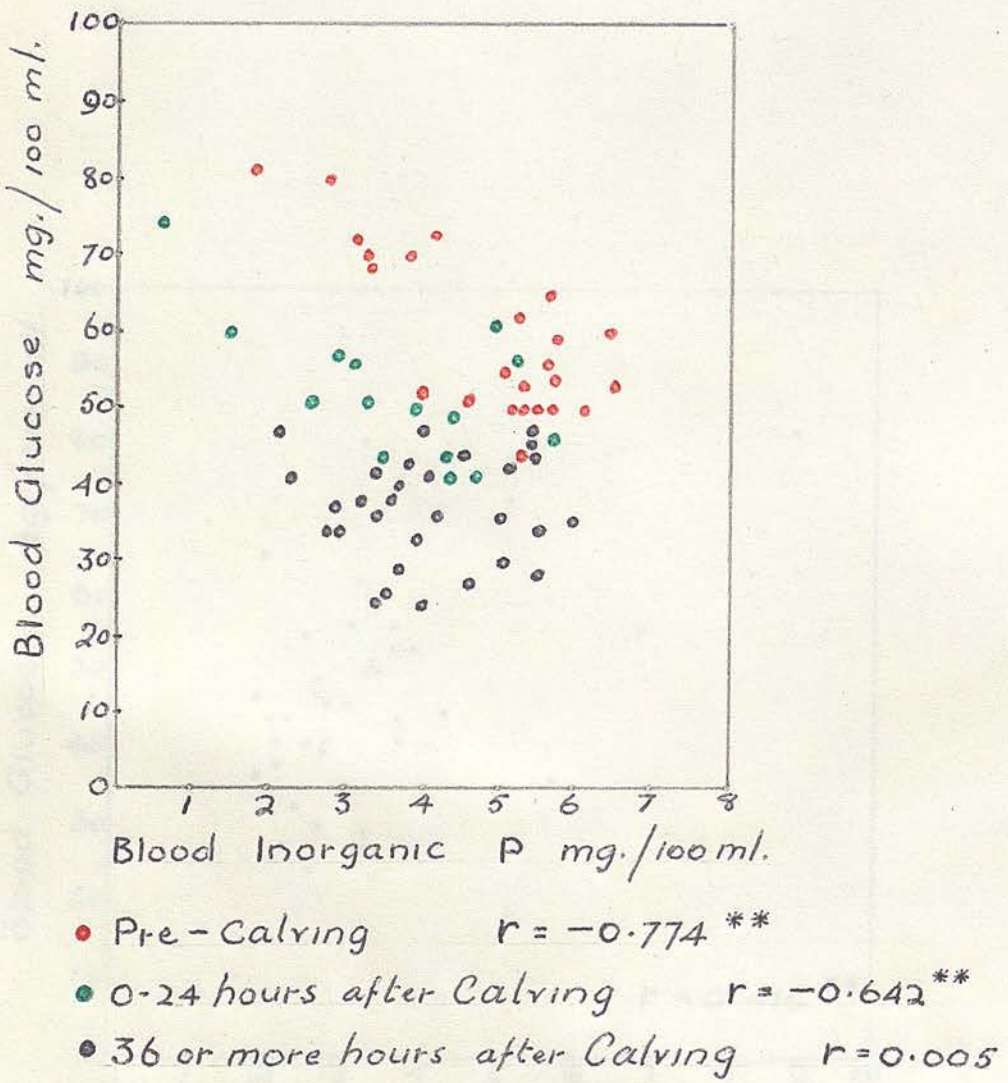


FIGURE 30 Correlations between the concentrations of glucose and inorganic phosphate in the blood of cows before and after calving.



citric acid for the individual cows differed significantly. Inspection of Figure 28 indicates some uniformity in the regressions for the four young cows, but in the old cows considerable variation was observed.

Lactic acid concentrations in the blood increased only transiently, while the glucose levels increased for a period of two or three days before calving. Nevertheless, the scatter diagram (Figure 29) shows a significant correlation.

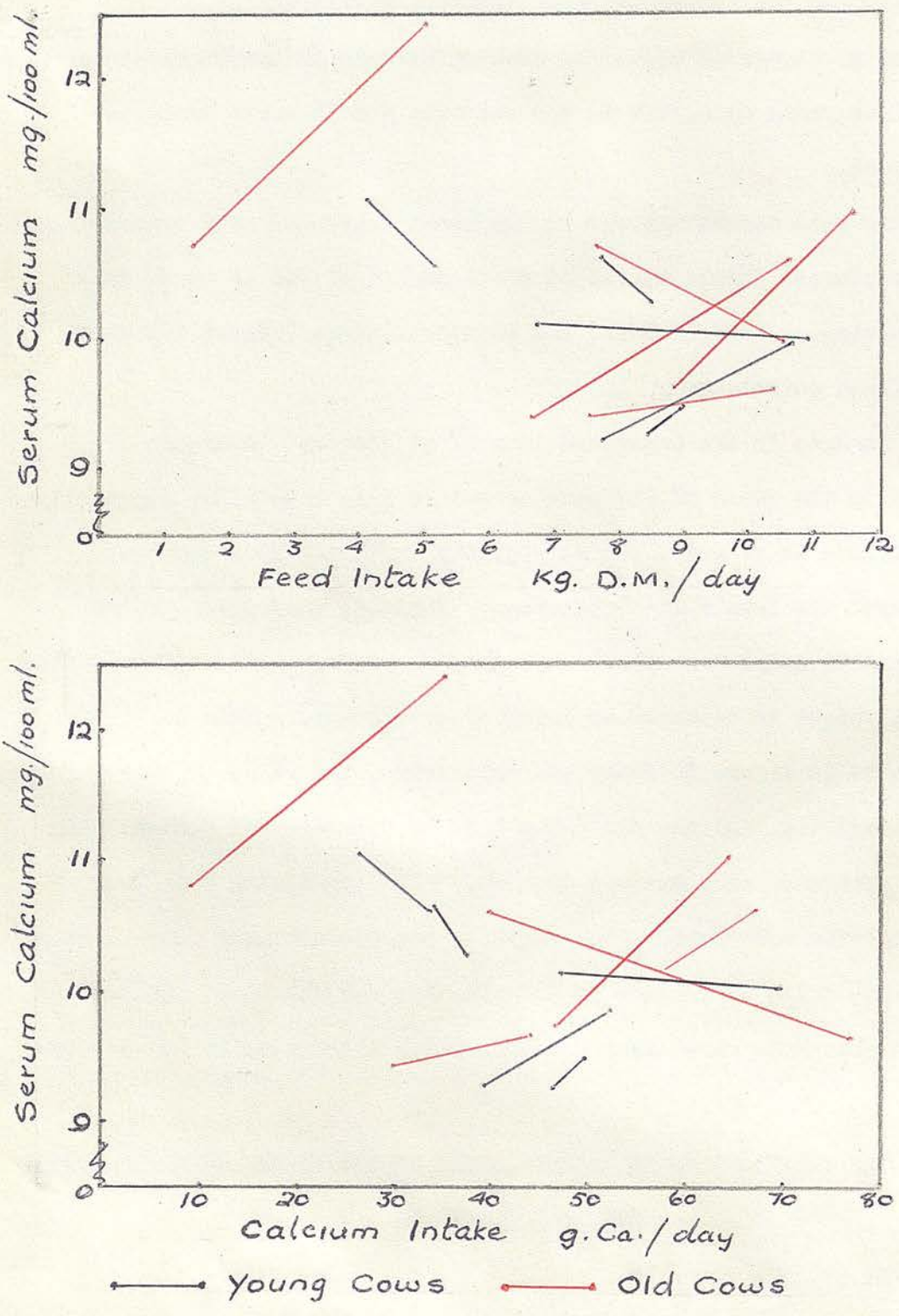
The changes in the concentrations of glucose and inorganic phosphate in the blood of the cows seemed to vary inversely, especially when allowance is made for the possibility that milk secretion may have lowered the levels after calving. However, inspection of the data from individual cows shows that this inverse relationship persisted only until eight to twenty-four hours after calving. This is demonstrated in Figure 30 where the precalving, the eight to twenty-four hour post-calving, and the one and a half to fourteen day post-calving data are presented as a scatter diagram. The precalving data show a high negative correlation, the eight to twenty-four hour post-calving data are not quite so regular in distribution and the blood glucose and inorganic phosphate concentrations thereafter altered quite independently.

Correlations and regressions between blood constituents, nutrient intake and milk secretion

(a) Before calving

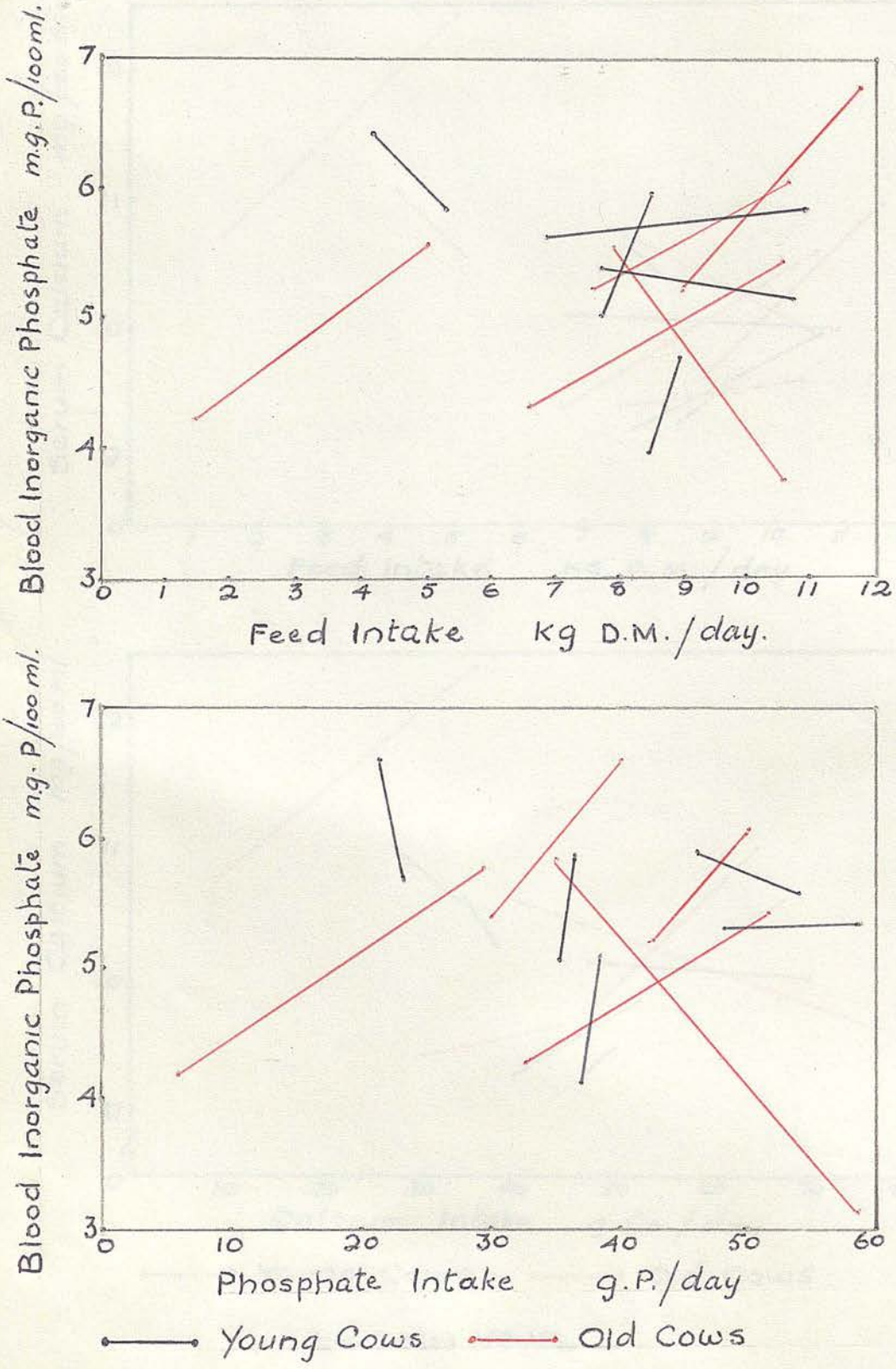
All the data obtained from the ten cows from ten days to one day

FIGURE 31 Regressions of serum calcium on feed intake and calcium intake of young and old cows before calving.



From Tables A22-A24.

FIGURE 32 Regressions of blood inorganic phosphate on feed intake and phosphate intake of young and old cows before calving.



From Tables A22, 25 and 26.

before calving (Table A22) was subjected to analyses of covariance to determine the regressions of serum calcium and blood inorganic phosphate on feed intake, of serum calcium on calcium intake, and of blood inorganic phosphate on phosphate intake. Full statistical details are given in Tables A23 - A26.

Figure 31 shows the individual regression lines for the ten cows of serum calcium on feed intake and of serum calcium on calcium intake. The positions of the regression lines are widely scattered, denoting marked differences between the cow means. These differences are significant. The slopes of the lines also differ significantly and so it is not possible to obtain an average regression of serum calcium on feed intake or calcium intake before calving. In the figure the lengths of the lines indicate the range of feed or calcium intake values from which the regressions were calculated.

Exactly the same picture is shown in Figure 32, where blood inorganic phosphate concentrations are compared with feed intake and phosphate intake.

(b) <u>After calving</u>

After calving the additional factor of milk yield can be readily measured and Table VII shows:

(a) the correlations between milk yield and feed intake, calcium intake and phosphate intake;

(b) the correlations between serum calcium and feed intake, calcium intake and milk yield and

TABLE VII

After calving correlations between feed, calcium and phosphate intake, serum calcium, blood inorganic phosphate and milk yield for all (total) data, for cows and within cows.

Correlation between	Total	Cows	Within Cows
Milk yield and:-			
feed intake	0.649**	0.686*	0.574**
calcium intake	0.455**	0.409	0.574**
phosphate intake	0.543**	0.557	0.513**
Serum calcium and:-			
feed intake	0.255	0.023	0.607**
calcium intake	0.268	0.045	0.602**
milk yield	0.083	-0.282	0.673**
Blood inorganic P and:-			
feed intake	0.374**	0.324	0.453**
phosphate intake	0.286*	0.201	0.451**
milk yield	0.186	0.097	0.327*

From Tables A27 - A30.

(c) the correlations between blood inorganic phosphate and feed intake, phosphate intake and milk yield.

These calculations are based on data collected from calving until seven days after calving. As with the precalving data, there were wide differences between the cow means, so in addition to the correlations on the total data, the table gives the correlations for the cow means and the average correlations within cows.

Attention is drawn to two features in the table. First, the correlations for total data for cows and within cows may or may not agree. Thus, the correlations between milk yield and feed, calcium, and phosphate intakes are almost identical whether calculated for total data, for cows or within cows, but the correlations between serum calcium and feed intake, calcium intake or milk yield are low for total but highly significant within cows. Secondly, the various correlations between milk yield, nutrient intake and blood constituent seem to be interdependent, as might be expected; within cows, feed intake and serum calcium, serum calcium and milk yield, and milk yield and feed intake are all significantly correlated.

Partial correlation and regression coefficients were therefore calculated, based on the following groups of three variates:

(a) feed intake, milk yield and serum calcium;

(b) calcium intake, milk yield and serum calcium;

(c) feed intake, milk yield and blood inorganic phosphate

and

(d) phosphate intake, milk yield and blood inorganic phosphate.

TABLE VIII

After calving partial correlations between feed, calcium and phosphate intakes, serum calcium, blood inorganic phosphate and milk yield for all (total) data, for cows and within cows.

Correlation between	Total	Cows	Within Cows
Milk yield and:-			
feed intake (a)	0.651**	0.722	0.281
	0.635**	0.695	0.505**
calcium intake	0.451*	0.441	0.286
phosphate intake	0.521**	0.553	0.414*
Serum calcium and:-			
feed intake	0.261	0.309	0.365*
calcium intake	0.259	0.183	0.356
milk yield (b)	-0.111	-0.409	0.499**
	-0.045	-0.329	0.498**
Blood inorganic P. and:-			
feed intake	0.339*	0.356	0.342
phosphate intake	0.224	0.178	0.333
milk yield (c)	-0.081	-0.182	0.092
	0.039	-0.018	0.125

(a) Third variate - serum calcium for upper line
blood inorganic P. for lower line

(b) Third variate - feed intake for upper line
calcium intake for lower line

(c) Third variate - feed intake for upper line
phosphate intake for lower line

From Tables A27 - A30.

There were therefore duplicate estimates of the partial correlations between milk yield and feed intake, milk yield and serum calcium and milk yield and blood inorganic phosphate.

The partial correlations (Table VIII) between milk yield and feed intake for total data are quite high and probably indicate a general tendency for high milking cows to have good appetites. The correlations between milk yield and calcium or phosphate intake are, of course, largely determined by the milk yield - feed intake correlation. Within cows, however, these correlations are low.

All the partial correlations between serum calcium and feed intake and between serum calcium and calcium intake are low except for the within cow correlations which are just significant at five per cent. The correlations between serum calcium and milk yield indicate that the slightly negative total correlation is made up of a positive within cow correlation and a negative correlation based on the cow means. This negative correlations is the only evidence obtained that the secretion of milk tended to lower the serum calcium levels.

No correlation worthy of comment was observed between blood inorganic phosphate and feed intake, phosphate intake or milk yield.

Table IX gives the partial regression coefficients, only a few of which are statistically significant. The data for cows indicate that high yielding cows ate more food, produced more milk (at the rate of 1.3 kilograms more milk per kilogram dry matter food eaten) and possibly had lower serum calcium levels than low yielding cows. On the other hand, within any cow a rise in serum calcium of one milligram per cent

TABLE IX

After calving partial regression coefficients between feed, calcium and phosphate intakes, serum calcium, blood inorganic phosphate and milk yield for all (total) data, for cows and within cows.

Regression	Total	Cows	Within Cows All	Within Cows Young	Within Cows Old
Milk yield (kg.) on:-					
feed intake (kg.)(a)	1.22***	1.29*	0.45	0.90	0.38
	1.22***	1.36*	0.92***	1.65**	0.71*
calcium intake (g)	0.14***	0.14	0.08	0.19	0.05
phosphate intake (g)	0.18***	0.19	0.17**	0.47*	0.13
serum calcium (mg.%)(b)	-	-	2.21***	1.90	2.24**
	-	-	2.10**	1.78	2.36*
blood inorganic P.	-	-	0.36	0.54	0.75
(mg.%) (c)	-	-	0.52	0.25	0.10
Serum calcium (mg.%) on:-					
feed intake (kg.)	0.12	0.12	0.13*	0.25*	0.10
calcium intake (g)	0.02	0.01	0.02*	0.05*	0.02
milk yield (kg.)(b)	-0.03	-0.09	-	-	-
	-0.01	-0.05	-	-	-
Blood inorganic P. (mg.%) on:-					
feed intake (kg.)	0.12*	0.44	0.16*	-0.14	0.19*
phosphate intake (g)	0.02	0.01	0.03*	-0.02	0.03
milk yield (kg.) (c)	-0.02	-0.04	-	-	-
	0.01	0.00	-	-	-

(a) Third variate - serum calcium for upper line
blood inorganic P. for lower line

(b) Third variate - feed intake for upper line
calcium intake for lower line

(c) Third variate - feed intake for upper line
phosphate intake for lower line

From Tables A27 - A38.

was associated with an increase in milk yield of about two kilograms per day, but as the partial correlation of the previous table was only 0.5, a rise in milk production could occur without any increase in serum calcium levels. The serum calcium within cows also tended to increase with feed intake at the rate of 0.13 mg. per cent for each kilogram of food eaten. Blood inorganic phosphate increased in the older cows with the improvement in appetite after calving, but this was not seen in young cows.

Discussion

Moodie, Marr and Robertson (1955) published the results of a survey on the serum calcium and plasma phosphate levels of normally calving cows in two herds. The serum calcium and whole blood inorganic phosphate levels from ten cows in a third herd in the same area are shown in Figure 26 and similar results were obtained, except that the phosphate levels failed to return to precalving values within fourteen days of calving. The changes in citric, lactic and pyruvic acids were similar to those reported by Ward et al., (1953b), while the changes in blood glucose agree with the reports of Fish (1927), Hayden and Fish (1927), van Soest and Blosser (1954) and Merrill and Smith (1954).

In Figure 26 the biochemical changes in the blood were divided into two groups - those where the maximum change was observed some hours after calving and those where the maximum occurred at calving. This natural separation of the data into groups tends to disprove the theory advanced by Ward et al., (1953b) that milk fever may be associated with impairment

of some of the oxidative decarboxylation processes of Kreb's cycle, since by the time the citric acid values were at their lowest the changes in the concentration of lactic acid in the blood were complete. Moreover, the lactic acid changes were much more transient that the citric acid changes. However, the division of the data into the two groups shown in Figure 26 does not necessarily imply complete independence of these chemical components from each other; there is, for example, considerable evidence of a link between phosphate and calcium metabolism in cases of milk fever (Robertson, Burgess, Marr and Milne, 1948; Robertson, 1949; Marr, Moodie and Robertson, 1955) and there is also evidence that both serum calcium and blood inorganic phosphate may continue to decline after calving in those cows developing milk fever (Marr, Moodie and Robertson, 1955; cow 5MF, this thesis). Glucose and lactic acid values also tend to be high in cases of milk fever and this could be due to the continuation of mechanisms already operating at calving.

Blosser and Smith (1950) reported that the changes in the serum calcium and citric acid concentrations in calving cows were similar and suggested that this might have some physiological significance. High concentrations of citric acid are found in the bones (Dickens, 1941) and are believed to be associated with the mobilisation of calcium, and there is also evidence that citric acid influences the absorption of calcium from the alimentary tract (although this is based on experiments in dogs where the circulating citric acid levels were raised far beyond their normal physiological range (Chang and Freeman, 1950; Freeman and

Chang, 1950a and 1950b)). However, Figure 27 indicates that there was no close similarity between the concentrations of calcium and citric acid in the blood of young cows. The regression coefficients within cows, depicted in Figure 28, also differed significantly, so there is probably no physiological significance attached to any apparent correlation between calcium and citric acid in the blood of calving cows. This view is supported by the wide range of citric acid values which may be obtained from cows with normal serum calcium values.

The glucose and inorganic phosphate concentrations in the blood of calving cows (Figure 30) were negatively correlated until calving, but no correlation was found from twenty-four hours after calving. This observation differs slightly from that of van Soest and Blosser (1954), where no correlation was found for normal cows at calving, although a correlation of -0.84 was obtained for milk fever cows. The rise in blood glucose levels at calving was accompanied by an increase in blood lactic acid (Figures 26 and 29), so there is no evidence that the low blood inorganic phosphate levels affected the conversion of glucose to lactic acid.

Until the significance of transient changes in blood inorganic phosphate level is established it may not be possible to suggest the circumstances which cause or permit a rise in blood glucose and lactic acid to coincide with a fall in blood phosphate levels. One might suggest that the high blood glucose and low inorganic phosphate levels were the result of reduced feed intake, especially as both these changes

were more marked in old cows than in young cows, but the work of Robertson, Paver, Barden and Marr (1960), indicates that changes in blood glucose of the magnitude shown by these calving cows would require several days of complete starvation and they did not find any reduction in blood phosphate levels until after realimentation. On the other hand, high blood sugars and reduced frequency and amplitude of rumen movements have often been associated (Le Bars, Nitescu and Simmonet, 1953; Alexander, 1954; Vallenas, 1956; Bowen, 1963) and there is a possibility that stasis of the alimentary tract may also be associated with lowered blood phosphate levels, a point which will be discussed more fully later.

In the previous section the suggestion was made that a failure in the absorption of nutrients due to stasis of the alimentary tract would be more important than the actual loss in nutrient intake, in influencing serum calcium and plasma phosphate levels. The failure to establish any uniform correlation in the before-calving data between feed, calcium or phosphate intakes, and serum calcium or blood inorganic phosphate concentrations does not affect this view, as the homeostatic mechanisms would probably be able to maintain blood calcium and phosphate levels in such non-lactating animals. Moreover, young cows can continue to eat normally even when gut activity is reduced, as indicated by their reduced faecal output (page 43). Clearly there is a limit to the volume of food that can be consumed under these conditions and by the time calving is complete the feed intake probably reflects more accurately the state of activity of the gut, which may account for the

better agreement among the individual cow regressions for serum calcium on feed intake after calving.

It was certain from inspection of the data that positive correlations between feed intake (and consequently calcium and phosphate intakes), serum calcium and blood inorganic phosphate, and milk yield would be found (Table VII), since all increased together after calving. Nevertheless, it is equally clear that the secretion of milk must drain calcium and phosphate from the blood and this must ultimately be replaced from the intestine, but it was not possible to measure this exchange in these experiments. The only evidence we have of this drainage is the negative partial regression among cows of 0.05 mg. calcium per 100 ml. serum for each kilogram of milk.

The within cow regression of two kilograms milk yield per milligram change in serum calcium concentration might suggest that the milk yield is controlled to some extent by the amount of calcium in the blood. However, other milk precursors in the blood are low at the same time, for example, citric acid and perhaps also protein (Vigue, 1952; Larson and Kendall, 1957), and all these blood constituents could be influenced by the appetite of the animal. It is, therefore, impossible to say at this stage if milk yield is directly influenced by the composition of the blood or whether appetite and milk yield are influenced by a common control mechanism, with the changes in blood composition being secondary.

Whatever the controlling mechanism may be, the net result is that

any improvement in food or calcium intake during this period is normally associated with a rise in milk yield and serum calcium concentration, and it is interesting to note that the addition of calcium as bone meal to a calcium deficient diet may improve the total lactation yields of cows (Becker, 1934; Arnold and Becker, 1936). The loss of calcium from the blood into the milk of the freshly calved cow is normally more than compensated for by mobilisation of calcium, otherwise serum calcium levels and milk yields would not rise together, but the partial correlations are not very high which indicates that high milk yields could occur where feed intake or serum calcium levels are falling. This situation could be expected to arise in the freshly calved cow as a result of maternal stimuli.

The blood inorganic phosphate levels of the old cows after calving also seemed to rise with food intake, but there was no evidence of a correlation between milk yield and blood phosphate. This is not surprising since the blood phosphate changes at calving in mastectomised cows are similar to those found in entire cows (Neidermeier, Smith and Whitehair, 1949; Robertson, Marr and Moodie, 1956). The correlation between blood inorganic phosphate and feed intake in young cows tended to be negative, because blood phosphate levels rose rapidly in young cows a few hours after calving and then declined. The reason for this change in blood phosphate level in young cows is not known, but apparently it is not the result of a surge in appetite and it is doubtful if the changes in blood inorganic phosphate in either young or old cows

are dependent on feed or phosphate intake.

Summary

Blood samples were collected at frequent intervals from ten cows before and after calving. Serum calcium and blood inorganic phosphate was estimated in all samples, blood citric acid in samples from eight cows, glucose and pyruvic acid in samples from five cows and lactic acid in samples from four cows. The concentrations of serum calcium and blood citric acid fell at calving and reached their lowest values about sixteen hours after calving, but blood inorganic phosphate reached its lowest value just at calving. Glucose and lactic acid concentrations increased to reach their maximum values at calving and no significant changes were observed in pyruvic acid.

A detailed study of the calcium and citric acid data indicates no physiological significance in the similarity of their concentration changes in serum and blood. The citric acid and lactic acid data indicate no impairment in the functioning of Kreb's cycle in these animals, and there would also seem to be no interference with the conversion of glucose to lactic acid. The changes in blood glucose and inorganic phosphate were negatively correlated until about one day after calving and were not thought to be the result of inanition.

An attempt was made to correlate serum calcium and blood inorganic phosphate changes with feed, calcium or phosphate intake and milk yield in the freshly calved cow. No significant correlations were found before calving between feed or calcium intake and serum calcium, nor

between feed or phosphate intake and blood inorganic phosphate. After calving, however, there was evidence that the cows with high milk yields ate more food and may have had slightly lower serum calcium levels than low yielding cows, but within any one cow the levels of feed intake, serum calcium and milk yield were positively correlated, the correlation between serum calcium and milk yield being the highest. There was a significant correlation between blood inorganic phosphate and feed intake only in the old cows.

SECTION IV

THE RELATIONSHIP BETWEEN ALIMENTARY ACTIVITY AND BIOCHEMICAL CHANGES IN THE BLOOD

It is now necessary to test experimentally some of the arguments advanced in the previous sections, in particular the suggestion that stasis of the alimentary tract can produce hypocalcaemia in the milking cow. This suggestion will be confirmed if it is shown that experimental hypocalcaemia has no immediate effect on alimentary activity and that experimentally induced alimentary stasis causes hypocalcaemia. In the experiments described in this section, hypocalcaemia was induced by sodium oxalate administration and alimentary stasis by hyoscine.

Materials and Methods

Animals:- The animals were drawn from the herd maintained at the Veterinary Field Station of the Royal (Dick) School of Veterinary Studies. This herd is managed as a normal commercial herd.

Sampling, Analytical and Recording Methods:- These were as described in the previous sections. Serum magnesium was estimated by the method of Denis (1922).

Results

(1) The effect of sodium oxalate injections

Two cows were given sodium oxalate by intravenous drip. Cow B52

TABLE X

Effect of sodium oxalate given by intravenous drip to two cows

	Cow No.	Before Injection	After Half Injection	End of Injection	After Calcium †
Serum calcium (mg./100 ml.)	B52	10.8	8.6	6.2	15.1
	E16	10.2	8.7	2.8	-
Serum magnesium (mg./100 ml.)	B52	2.70	2.70	2.55	2.40
	E16	2.50	2.50	2.50	-
Blood inorganic phosphate (mg./100 ml.)	B52	2.82	2.88	2.75	3.08
	E16	3.81	3.97	3.63	-
Blood citric acid (mg./100 ml.)	B52	1.95	2.14	2.37	2.39
	E16	2.95	2.95	3.60	-
Rumen sounds score	B52	2.0	2.0	2.0	2.00
	E16	2.5	2.5	2.5*	-
Total rumen movements/10 min.	B52	18	17	15	14
	E16	22	18	16*	-
Primary rumen movements/10 min.	B52	10	9	8	7
	E16	13	11	10	-
Secondary rumen movements/10 min.	B52	8	8	7	7
	E16	8	7	7	-

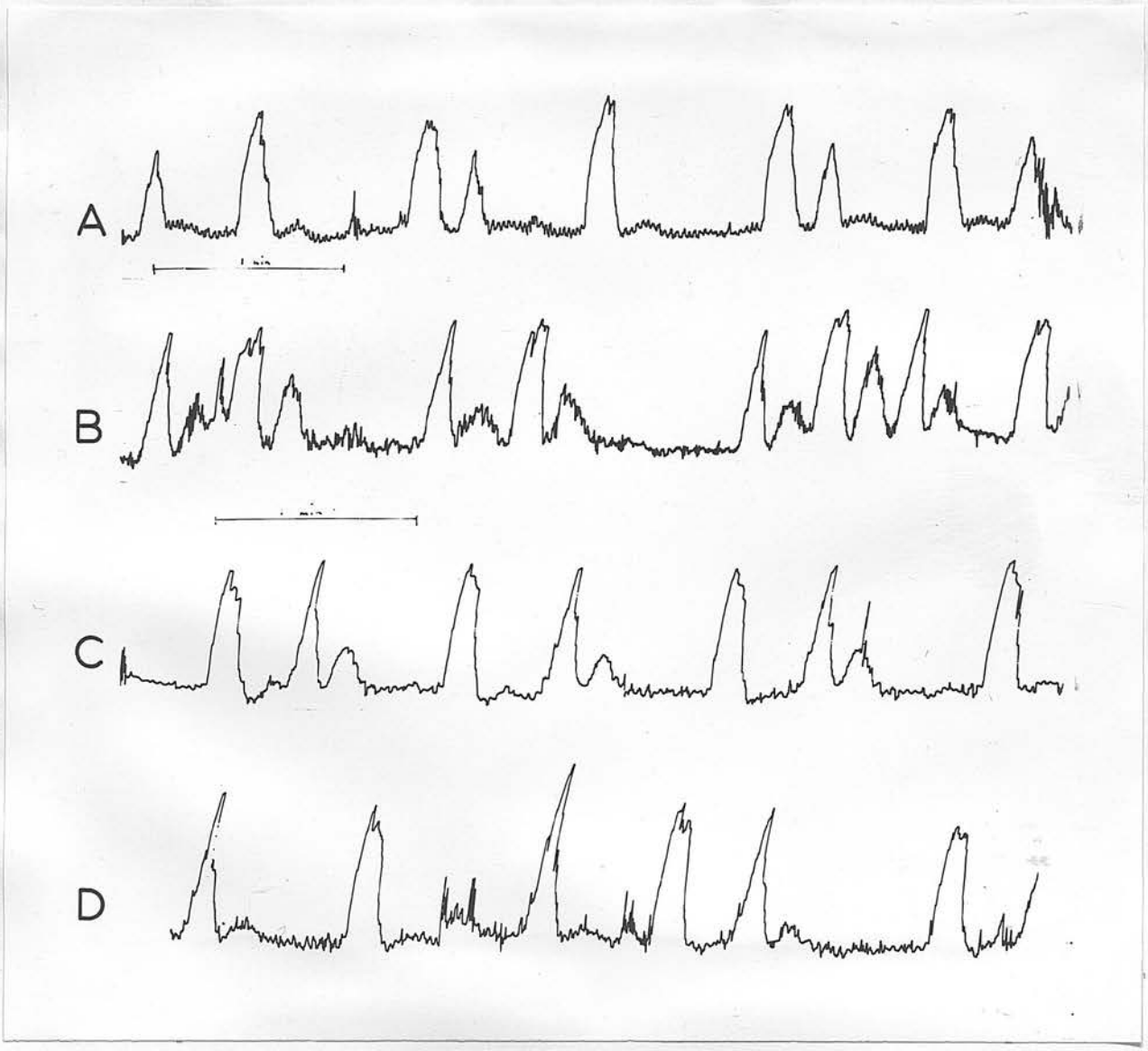
* five minutes before end of injection.

† ten minutes after the intravenous administration of 80 g. calcium borogluconate at the end of the oxalate injection.

B52 received 16 g. sodium oxalate as a two per cent solution in 60 minutes.

E16 received 19 g. sodium oxalate as a two per cent solution in 55 minutes.

FIGURE 33 Rumen movements recorded from the left paralumbar fossa of cow B52 during the intravenous injection of 16 g. sodium oxalate in one hour.



A - before oxalate injection

B - half hour after commencement of oxalate injection

C - end of oxalate injection

D - ten minutes after 80 g. calcium borogluconate i/v

FIGURE 34 Rumen movements recorded from the left para lumbar fossa of cow E16 which died following the intravenous injection of 19 g. sodium oxalate in 55 minutes.

A - before oxalate injection

B - half hour after commencement of oxalate injection

C - 53-57 mins. after commencement of oxalate injection

D - point of death, 57-60 mins. after commencement of oxalate injection

(dry and barren) received 16 g. sodium oxalate as a two per cent solution evenly over a period of sixty minutes and cow E16 (dry, seven months pregnant) died after 19 g. sodium oxalate had been administered in fifty-five minutes. In cow B52 the oxalate reduced the serum calcium level by 2.2 mg. per cent in the first thirty minutes and by 2.4 mg. per cent in the second thirty minutes (Table X). In cow E16 the corresponding figures were 1.5 and 5.9 mg. per cent. The changes were not associated with any change in serum magnesium or blood inorganic phosphate values but the citric acid values tended to increase slightly. Rumen sounds scores remained unaltered at 2.0 and 2.5 for cows B52 and E16 respectively and the frequency of the rumen movements also did not alter.

Figures 33 and 34 show tracings of the rumen movements of the cows before the injection, after half the injection and on the completion of the injection. No change was noticed except in cow E16 at the point of death. Figure 33 also shows the recording of the rumen movements of cow B52 after the administration of eighty grams of calcium borogluconate intravenously at the end of the oxalate infusion. This injection raised the serum calcium from 6.2 to 15.1 mg. per cent ten minutes later, but apparently had no effect on the rumen activity.

(2) The effect of hyoscine hydrobromide

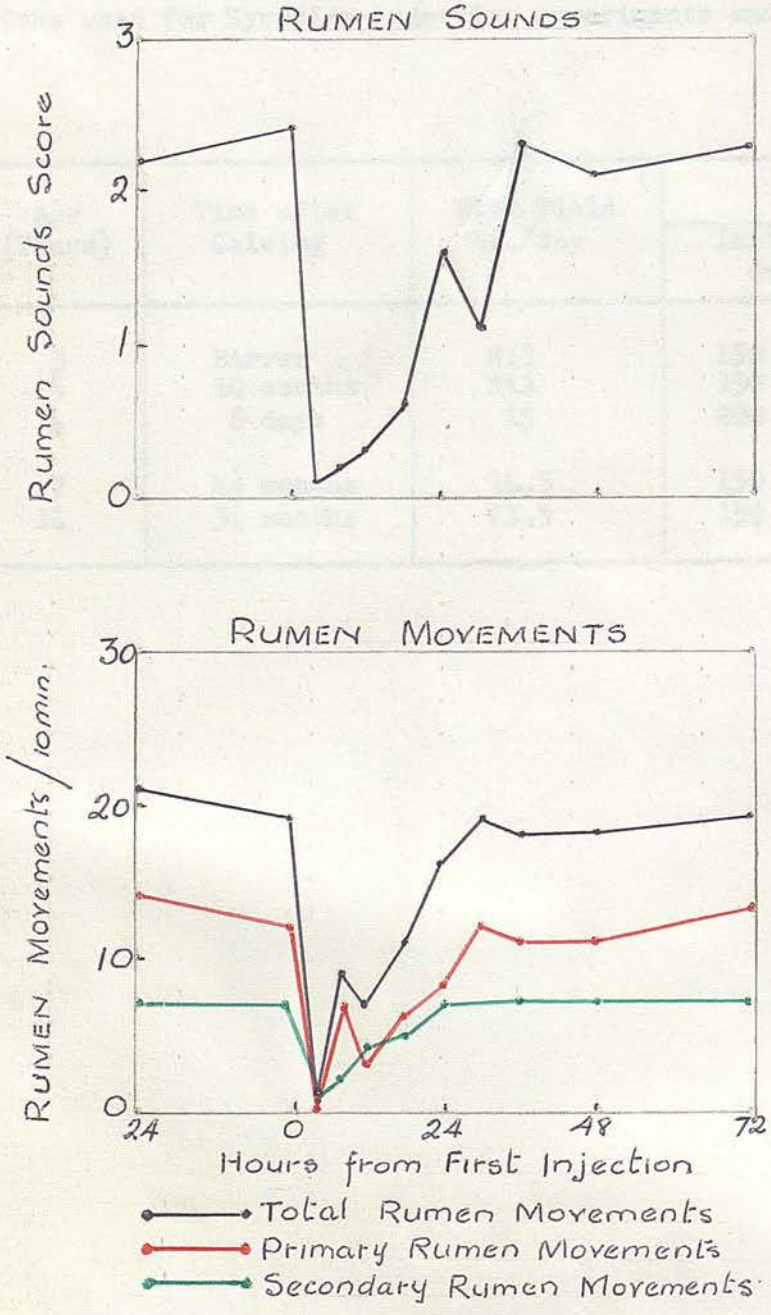
Five cows were injected subcutaneously with hyoscine hydrobromide B.P. with the object of inhibiting peristalsis, as indicated by the frequency of defecation, for a period of at least sixteen hours.

TABLE XI

Cows used for Hyoscine injection experiments and dose rates

Cow No.	Age (Years)	Time after Calving	Milk Yield kg./day	Hyoscine hydrobromide	
				Initial dose	Maintenance doses
F4	3	Barren	Nil	150 mg.	50 mg. after 12 hrs
D19	5	10 months	Nil	150 mg.	50 mg. after 12 hrs
E14	4	8 days	15	200 mg.	100 mg. after 8 hrs 50 mg. after 12 hrs
B51	7	$4\frac{1}{2}$ months	14.5	150 mg.	50 mg. after 10 hrs
P11	11	$3\frac{1}{2}$ months	21.5	150 mg.	Nil

FIGURE 35 The effect of hyoscine hydrobromide administered subcutaneously on the rumen sounds and movements of five cows.



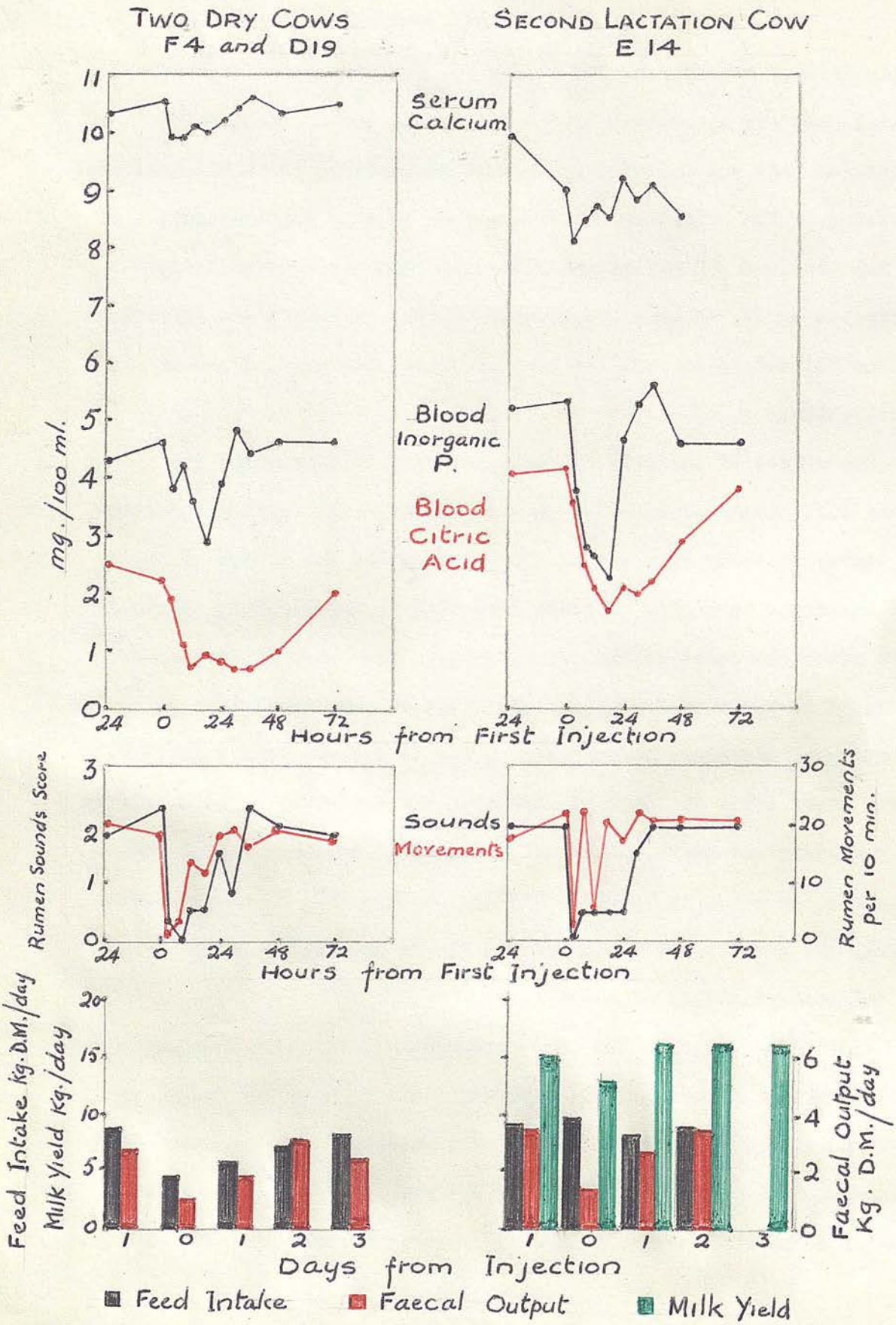
From Table A39.

Particulars of animals and doses used are given in Table XI. The initial dose was approximately 0.5 milligrams per kilogram body weight and this was followed by smaller maintenance doses as considered necessary. The effect of the drug seemed to vary considerably. Cow E14 received 350 milligrams altogether and was comparatively unaffected by it, while a single dose of 150 milligrams was sufficient for cow P11 and in cow B51 the drug produced scouring instead of constipation.

The effect of the drug on rumen activity is summarised in Figure 35. Rumen sounds disappeared following the initial injection but improved slowly at first and then rapidly as the effect of the last injection wore off. Sounds were back to normal about thirty-six hours after the start of the experiments. The rumen movements also decreased markedly in frequency and amplitude immediately following the injection. Secondary movements appeared to recover more quickly and thirty hours after the initial injection the frequency of both primary and secondary movements was normal. Selected tracings of the rumen movements are given in appendix II (Figures A1 - A5), which show quite clearly the greater resistance of cow E14 to the action of the drug and the susceptibility of cow P11.

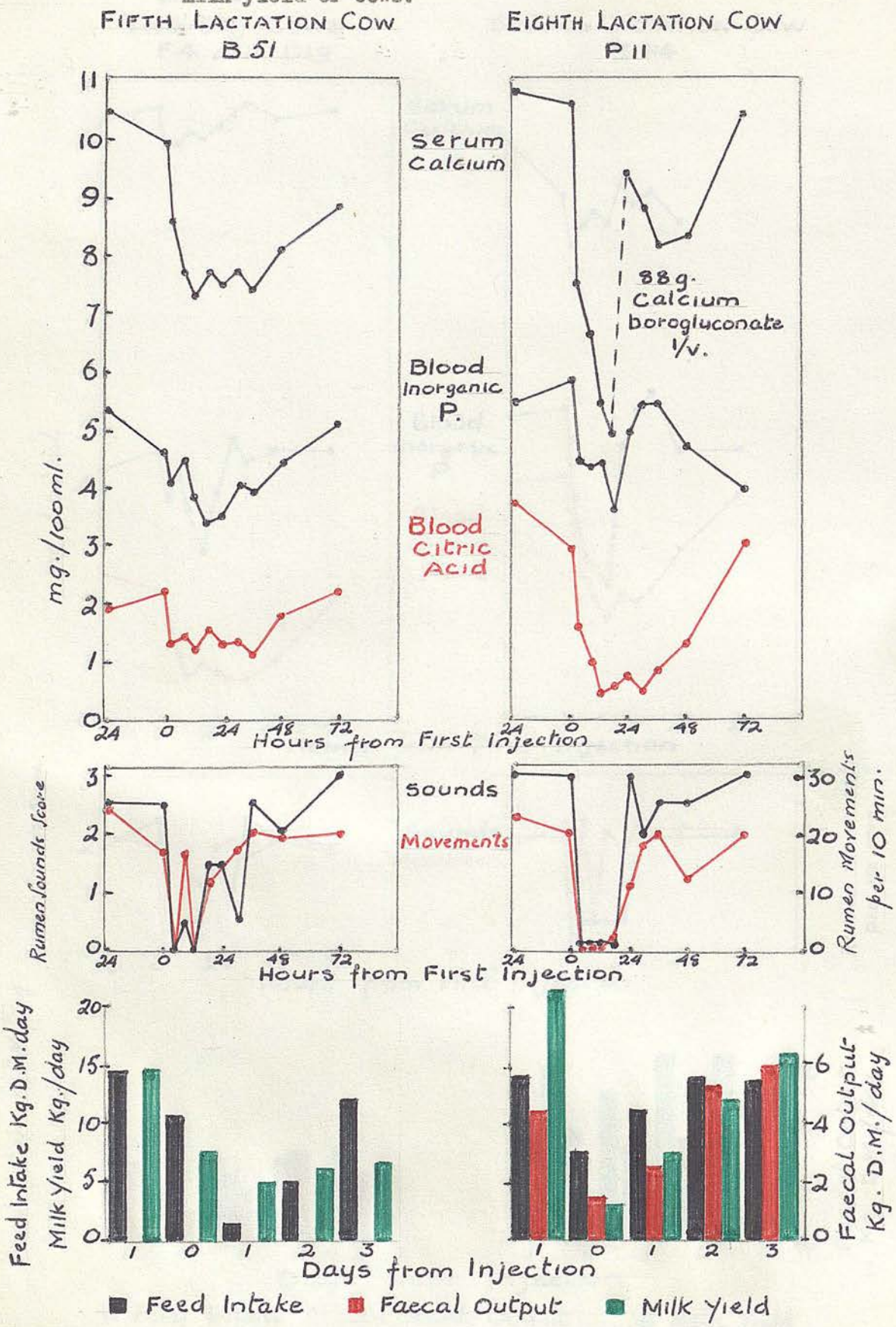
The initial dose of the drug produced mild bloating which persisted for two or three hours. This bloating diminished as the secondary movements returned to normal and was not observed with the succeeding maintenance injections. Salivation was also inhibited, but despite this the animals attempted to eat as soon as the more severe effects of

FIGURE 36 Effect of hyoscine hydrobromide injections on blood constituents, alimentary activity and milk yield of cows.



From Tables A39-A41.

FIGURE 37 Effect of hyoscine hydrobromide injections on blood constituents, alimentary activity and milk yield of cows.



From Tables A39-A41.

the drug wore off, but before the return of the rumen activity or salivation to normal.

The effect of the treatment on the appetite, faecal output and milk yield and on the serum calcium and blood inorganic phosphate and citric acid levels of the cows is shown in Figures 36 and 37. The data from the two dry cows were similar and have been combined into one diagram, but the data from the milking cows are shown individually. In four animals the most severe changes were noted on the day of injection, but in cow B51 the maximum effect was on the day following the injection.

The effect of the treatment was to reduce the average food consumption of the animals by thirty per cent, but for the actual period of the inoculations the inappetence was virtually complete. One cow, E14, recovered in time to eat her total ration within the twenty-four hour sampling period. The calcium and phosphate intakes are not shown shown in the figures, but are recorded in Table A40. These follow the changes in feed intake quite closely. Faecal output fell on the day of injection to between one half and one third of normal in four of the cows (collection was impossible from B51 which scoured). Milk yield dropped by fifteen per cent in cow E14 on her second lactation, fifty per cent in cow B51 and eighty-seven per cent in cow P11.

Blood citric acid dropped sharply following the first injection and the change was most marked where the initial citric acid level was high. The low levels persisted for about thirty-six hours and the return to

normal values was gradual. Blood inorganic phosphate values declined a little more gradually and reached their lowest values about sixteen hours after the first treatment, but the levels rose rapidly to normal following the disappearance of the effects of the hyoscine treatment.

The serum calcium levels of the dry cows dropped by about 0.5 mg. per cent for the day of the injections and a similar change was observed in the young milking cow. The older cows showed more marked changes, cow B51 on her fifth lactation showing values of about 7.5 mg. per cent for twenty-four hours and cow P11 (eighth lactation) a rapid drop in serum calcium to a value of 4.9 mg. per cent at sixteen hours after the injection. At this time the cow was recumbent, her rumen movements and sounds were not detectable and she displayed all the signs typical of milk fever. This cow recovered about six hours after the intravenous administration of calcium borogluconate.

Discussion

The administration of sodium oxalate to two cows induced a mild hypocalcaemia in thirty minutes and a moderate or severe hypocalcaemia in sixty minutes. There was no evidence of any alteration in the rumen activity of these two animals, except during the terminal stages in the animal which died and, as in the case of those sheep in Section V which died following oxalate injections, the death was unheralded and quite atypical of the milk fever syndrome. There was also no change in the rumen activity of the other cow following the administration of calcium borogluconate solution sufficient to produce hypercalcaemia. These

results would seem to indicate that a mild hypocalcaemia of the type often found in normal calving cows, where the serum calcium remains above 8 mg. per cent, does not induce stasis of the rumen. This view is supported by the persistence of rumen and alimentary tract activity in cow B51, which was injected with hyoscine and had a serum calcium level of about 7.5 mg. per cent for twenty-four hours. A similar opinion was expressed by Payne (1964) who carried out prolonged EDTA injections on cows.

However, Hallgren, Carlstrom and Jonsson (1959) have reported some alimentary dysfunction in two of four cows where sodium oxalate was administered for several hours, so a prolonged hypocalcaemia might induce alimentary stasis. These four cows had similar serum calcium values at the end of the experiment. Also, clinical cases of hypocalcaemia often defecate following the administration of calcium salts, but this does not necessarily prove that the stasis was caused by hypocalcaemia. If severe hypocalcaemia does interfere with gut activity it would account for the low dose of hyoscine necessary to induce alimentary stasis in cow P11 which developed milk fever.

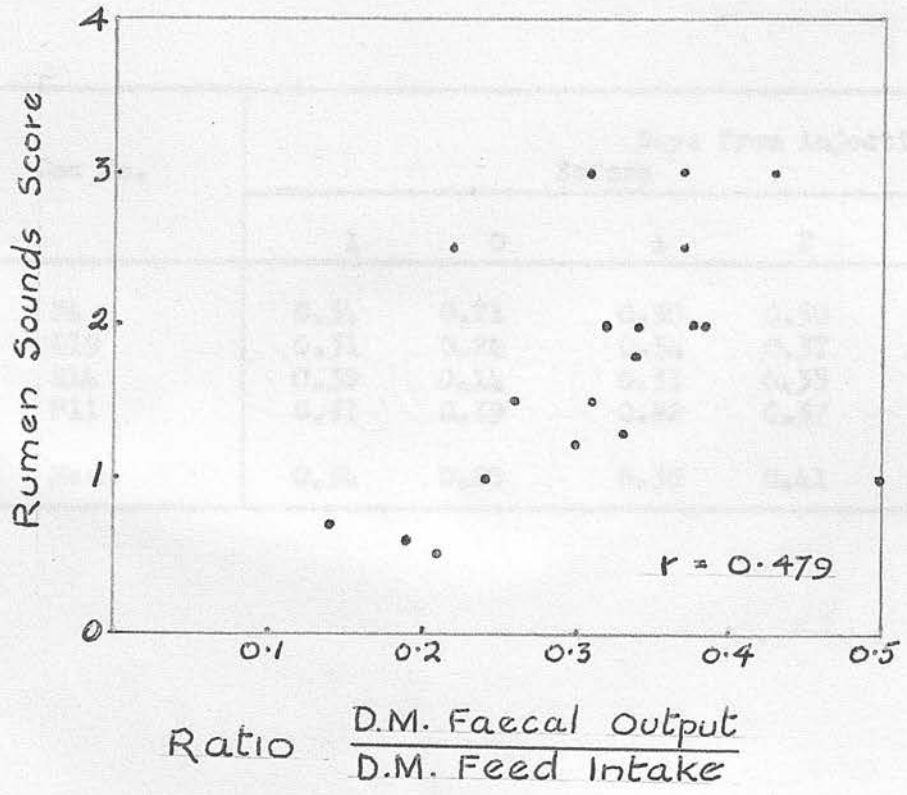
On the other hand, there is little doubt that alimentary stasis can produce a hypocalcaemia under certain circumstances. In the non-milking cows the changes in serum calcium were slight and rather similar to those observed in mastectomised cows (Neidermeier, Smith and Whitehair, 1949; Robertson, Marr and Moodie, 1956), while the change in the serum calcium of the cow at her second lactation resembled that seen in cows at their second calving. The serum changes in the older cows which were milking

TABLE XII

Ratio between the dry matter excreted in the faeces and the dry matter of the food consumed during 24 hours $\left(\frac{\text{D.M. Faeces}}{\text{D.M. Food}}\right)$ of four cows injected with hyoscine hydrobromide.

Cow No.	Days from injection				
	Before		After		
	1	0	1	2	3
F4	0.34	0.21	0.30	0.50	0.26
D19	0.31	0.24	0.34	0.37	0.32
E14	0.38	0.14	0.33	0.38	-
P11	0.31	0.19	0.22	0.37	0.43
Mean	0.34	0.20	0.30	0.41	0.34

FIGURE 38 Correlation between rumen sounds score and the ratio of dry matter faecal output to dry matter feed intake in cows injected with hyoscine hydrobromide.



heavily were more marked and occurred with a rapidity comparable to those observed in normal calving cows and in cows developing milk fever (Moodie et al., 1955; Marr et al., 1955). The other biochemical changes which were observed also resembled those seen in cows at calving; blood inorganic phosphate values fell following the hyoscine administrations and recovered as the alimentary stasis wore off, citric acid levels also fell but recovered more slowly and serum magnesium values which were obtained from three of the cows tended to rise (Table A41).

Calving cows and hyoscine treated cows also showed similar changes in alimentary activity and appetite. The reduction in feed intake of the hyoscine treated animals averaged thirty per cent, but the faecal output of the four cows where this was measured dropped by about sixty per cent. Thus the ratios of dry matter faecal output to dry matter feed intake changed by about the same extent as was found for the normal calving cows (Section II), so providing evidence of an accumulation of solids in the digestive tract (Table XII). During the period when the ratios of faecal output to feed intake were low, the rumen movements could often be recorded even though rumen sounds were absent, and the correlation between rumen sounds and this ratio was almost identical to that obtained for calving cows (Figure 38).

Scouring was produced in cow B51. This was associated with reduced rumen activity and appetite and these changes persisted over a longer period than in the other four animals. The hypocalcaemia was also more prolonged, so derangement of alimentary activity, other than stasis, can perhaps lead to hypocalcaemia and hypophosphataemia.

TABLE XIII

Within cow correlations, partial correlations and regressions for feed intake, serum calcium, blood inorganic phosphate and milk yield of calving cows and hyoscine treated cows.

	Correlation		Partial Correlation		Partial Regression	
	Calving Cows	Hyoscine Treated Cows	Calving Cows	Hyoscine Treated Cows	Calving Cows	Hyoscine Treated Cows
Milk yield (kg.) and:-						
feed intake (kg.)	0.574**	0.625*(a)	0.281	0.072	0.45	0.06
		(b)	0.505**	0.560	0.92***	0.77
serum calcium (mg.%)	0.673**	0.878**	0.499**	0.791*	2.21***	3.49*
blood inorganic P. (mg.%)	0.327*	0.373	0.092	0.175	0.36	1.12
Serum calcium (mg.%) and feed intake (kg.)	0.607**	0.683**	0.365*	0.359	0.13*	0.07
Blood inorganic P. (mg.%) and feed intake (kg.)	0.453**	0.396	0.342	0.225	0.16*	0.05

(a) Third variate - serum calcium

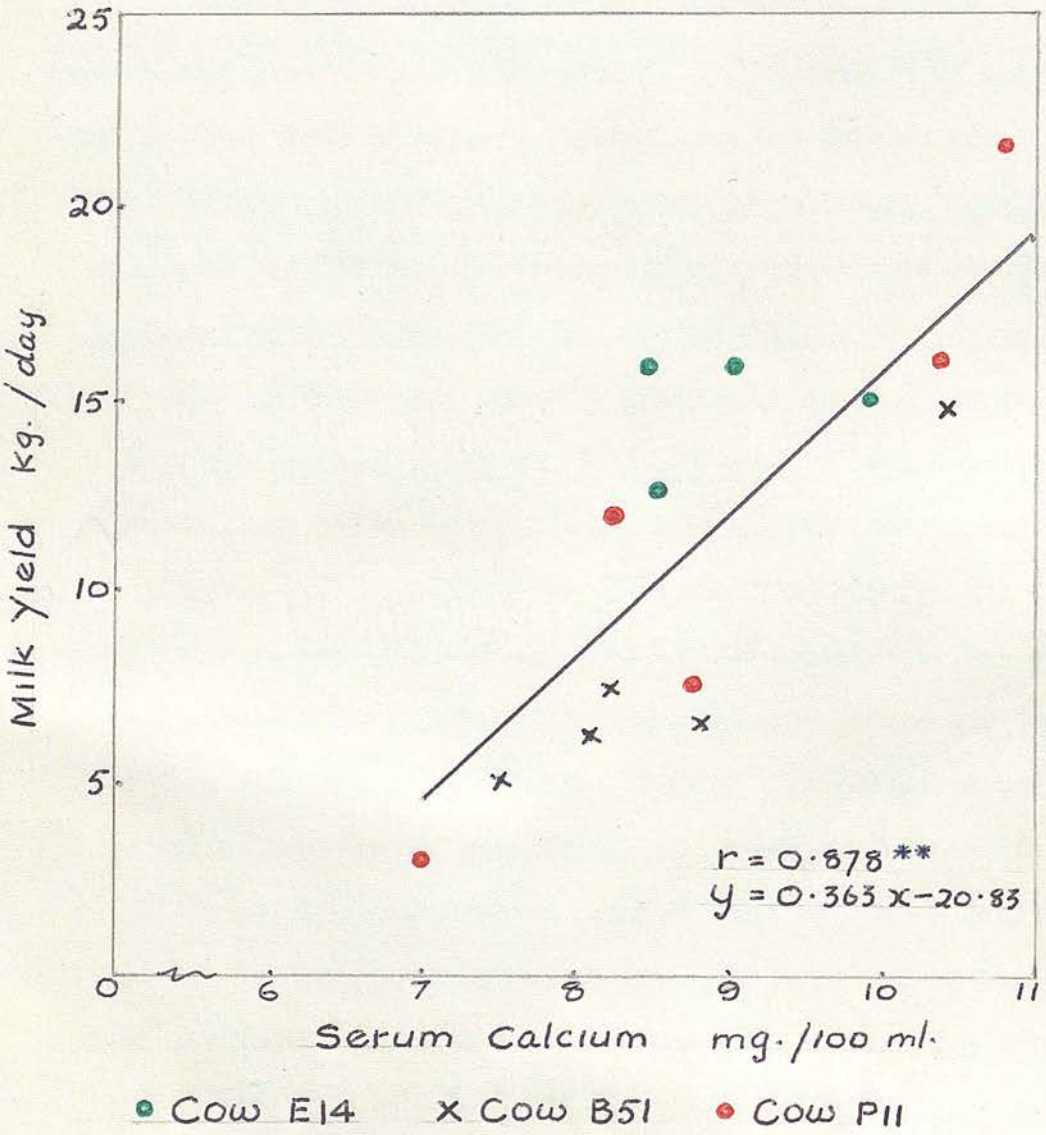
(b) Third variate - blood inorganic phosphate

From Tables VII, VIII, IX, A42 and A43.

These biochemical and physiological features, together with the development of typical milk fever symptoms in one animal, support the idea that alimentary stasis could be a cause of parturient hypocalcaemia in cattle.

Inspection of Figures 36 and 37 indicate a close association between milk yield, serum calcium and feed intake, similar to that observed for cows at calving. Accordingly, correlation coefficients, partial correlation coefficients and partial regression coefficients have been calculated for the three milking cows injected with hyoscine and the results are shown in Table XIII along with the corresponding data for normal cows, taken from Tables VII, VIII and IX. The similarity of the simple correlations for calving and hyoscine treated cows, shown in the first two columns of Table XIII, is striking. The partial correlations and regressions of the hyoscine treated cows also follow the trends of the normal cows but the levels of significance are lower because of fewer degrees of freedom. Nevertheless, it would seem that the milk yield was very closely correlated with the changes in serum calcium and that a drop in serum calcium concentration of one milligram per cent tended to be associated with a drop in milk yield of 3.5 kg. per day in the hyoscine treated cows. It would seem, therefore, that any effect of feed intake on milk yield in calving cows, discussed on page 67, is probably an indirect one through its effect on the serum calcium balance. As discussed previously, the regressions do not account for the balance between the loss of calcium from the serum to the milk and its replacement from the digestive tract or skeleton.

FIGURE 39 Regression of milk yield on serum calcium concentration in cows injected with hyoscine hydrobromide.



The regression equations of milk yield on feed intake and serum calcium levels for young and old calving cows and hyoscine treated cows are as follows:-

Young cows	$Y = 0.90X_1 + 1.90X_2 - 12.59$
Old cows	$Y = 0.38X_1 + 2.24X_2 - 6.04$
Hyoscine injected cows	$Y = 0.06X_1 + 3.49X_2 - 20.23$

where Y = milk yield (kg. per day), X_1 = feed intake (kg. D.M. per day) and X_2 = serum calcium concentration (mg. per cent). The corresponding multiple correlation coefficients are 0.634, 0.765 and 0.878. These indicate the extent to which the milk yield is correlated with the combined effect of feed intake and serum calcium level. The item $0.06X_1$ in the regression equation for hyoscine injected cows is so small that the equation may be simplified to:

$$Y = 3.63X_2 - 20.83$$

The values from these cows may now be shown graphically, as in Figure 39.

The lower multiple correlation coefficients for the freshly calved cows indicate that in these animals the milk yield was not so well correlated with the serum calcium levels and feed intake as in the hyoscine treated cows. In addition, the milk yield of the calving cows only changes by 1.9 and 2.2 kg. per day in young and old cows for each milligram per cent change in serum calcium concentration, compared with 3.5 kg. per day in the hyoscine treated cows. Thus a fall in serum calcium of three milligrams per cent was associated with an average reduction in milk yield of 10.5 kg. per day in the hyoscine treated cows, but only of about six kilograms in the freshly calved cows. This difference may represent a

stronger physiological requirement by the freshly calved cow to produce nourishment for her calf.

This adjustment of milk yield to serum calcium levels possibly acts as a partial defence mechanism to control hypocalcaemia. Thus in both calving cows and hyoscine treated cows, when milk yield was rising the supply of calcium into the circulation increased more rapidly than the drainage of calcium into the milk, otherwise serum levels would not have increased as they did following calving or during recovery from hyoscine medication. Conversely, the data indicate that when milk yields and serum calcium levels fall, the supply of calcium to the blood decreases more rapidly than the drainage of calcium into the milk. The interesting question is whether a point of balance between the uptake of calcium by the blood and loss of calcium from the blood can be reached before milk secretion ceases. If no point of balance is reached while the animal continues to milk, and if the regression of milk yield on serum calcium is the same for heavily and lightly milking cows, as indicated by the regression equations above for young and old cows which had different average milk yields, then the heavily milking cow will be at a serious disadvantage when faced with conditions predisposing to hypocalcaemia. No matter whether the cow is dry or is milking, her normal serum calcium level is about ten milligrams per cent, so if the above assumptions are correct a freshly calved cow yielding ten kilograms of milk per day would go dry when her serum calcium fell to five milligrams per cent, but a cow yielding twenty kilograms of milk per day would not go dry before death supervened.

The evidence that serum calcium levels and milk yield are correlated is not confined to cows, since Cowie and Folley (1945) have noted that lactation in rats was severely depressed following parathyroidectomy. It is not clear, however, whether calcium deficiency has a direct effect on the secretion of milk or whether it induces secretory changes in some of the endocrine organs. Douglas (1962; 1963) has demonstrated that calcium is important for sensitising various organs, including the posterior pituitary gland, to the effects of acetylcholine.

Table XIII also shows that the changes in the blood inorganic phosphate of normal cows or cows injected with hyoscine were not strongly associated with changes in feed intake or milk yield.

Neidermeier et al., (1949) and Robertson et al., (1956) have both shown that in mastectomised cows the serum phosphate levels fall at calving to about the same extent as in entire cows, so it seems that the bulk of the changes in blood inorganic phosphate at calving are not due to drainage into the milk. It may be that urinary excretion of phosphate at calving time is increased, although as yet there is no information available on this point, but a more likely route of phosphate loss from the blood is to the alimentary canal, especially the rumen. The loss of phosphate from the serum is similar in dry and milking cows injected with hyoscine and in normal calving cows and these losses in all cases appear to be cumulative, since the blood phosphate levels decline progressively so long as the alimentary stasis persists (Figures 26, 36 and 37). The possibility of accumulation of foodstuffs in the alimentary canal has already been discussed and this could be associated with a retention in

the digestive tract of the phosphate which is secreted through the saliva and the rumen wall. Smith, Kleiber, Black and Baxter (1955) estimate this combined secretion at 4.8 g. per day for ten month old sheep and the figure for cattle would be proportionately greater.

At first sight there appears to be some interaction between serum calcium and blood phosphate levels. Although no change in blood phosphate values was observed in the two cows injected with oxalate, Smith and Brown (1961) and Payne (1964) have reported that plasma inorganic phosphate concentrations fell one to two milligrams per cent during EDTA infusions and a similar effect is noted in the next section with oxalate infusions in sheep. However, the pattern of the changes in serum calcium and blood phosphate during oxalate infusion and normal calving seem to be quite different, since during oxalate infusion both blood constituents tended to change simultaneously, while at calving the main phosphate change preceded the main calcium change and in any event may be observed in mastectomised cows where the calcium changes are negligible. Thus the changes in blood phosphate on the day of calving can hardly be attributed to changes in serum calcium level and the same is true for those cows, which were injected with hyoscine, where little alteration in serum calcium was observed.

Citric acid levels also fell in the hyoscine injected cows but not in the oxalate treated animals and, as was observed in the normal calving cows, there was no relation between the serum citric acid and serum calcium changes in the individual animals. It may be that the citric acid changes were associated in some way with variations in the alimentary activity of

the cows. In the cows injected with hyoscine the lowest citric acids occurred from twelve to thirty-six hours after the initial dose, while in the calving cows the lowest values occurred from eight to thirty-six hours after the period of maximum alimentary stasis.

Summary

Sodium oxalate was administered intravenously to two cows at the rate of 16 g. in sixty minutes and 19 g. in fifty-five minutes. The animal which received the higher dose died at the end of the injection. Serum calcium concentrations fell to 6.2 and 2.7 mg. per cent respectively, but no changes were observed in serum magnesium, blood inorganic phosphate or citric acid concentrations. Rumen sounds score and rumen movements were unaffected, except terminally in the animal which died. No symptoms were produced by oxalate injection typical of milk fever in cattle.

Two dry cows and three milking cows were injected with hyoscine hydrobromide subcutaneously. In four cows the drug produced stasis of the bowel but the fifth cow scoured. The alimentary stasis was associated with loss of rumen sounds and movements, reduced faecal output and impaired appetite. Milk yield was reduced most markedly in the oldest and least in the youngest milking cow. Serum calcium changes were only slightly lowered in the dry cows and the youngest milking cow, but the two older milking cows showed low calcium levels and the eldest cow developed symptoms typical of milk fever. Inorganic phosphate and citric acid concentrations in the blood were reduced and serum magnesium concentrations were increased slightly. These physiological and biochemical changes are compared with

those occurring in calving cows.

A statistical treatment of the data shows that the milk yield of cows is highly correlated with the serum calcium levels. In freshly calved cows an increase in serum calcium of one milligram per cent was associated with a rise in milk yield of approximately two kilograms, but in non-parturient cows the corresponding rise in milk yield was three and a half kilograms. This finding is discussed in relation to the natural defence of the animal against hypocalcaemia.

SECTION V

THE PREVENTION OF HYPOCALCAEMIA

Most methods of preventing milk fever depend on augmenting the labile reserves of calcium in the animal or reducing the drainage of calcium from the blood into the milk. Greig (1930) first suggested the injection of calcium salts for the prevention of parturient hypocalcaemia, two injections with an interval of twenty-four hours being recommended, but there are apparently no records of the efficacy of this approach. However, milk fever may occur at any time over a period of four or five days and a therapeutic dose of calcium borogluconate (100 g. containing about 7 g. calcium) only persists in the circulation for six to ten hours (Moodie, 1952). Ward and Vair (1959) fed a calcium lactate - aluminium hydroxide compound to cows before calving but it is doubtful if any real benefit was obtained. Recently, Kendall and Harshbarger (1961) reported that the intra-peritoneal injection of 500 ml. of 27.3 per cent calcium alumino-gluconate thirty minutes after calving elevated serum calcium levels by an average of sixteen per cent over the next twenty-four hours and the peak effect seemed to be four to six hours after the injection.

Prepartum milking to produce a more gradual onset to the lactation and partial milking at the beginning of lactation have not efficiently prevented milk fever (Smith and Blosser, 1947; Neidermeier and Smith, 1948; Smith, Neidermeier and Hansen, 1948), although cessation of

milking later in the lactation is claimed to produce a rise in serum calcium levels (Mercer, Eaton, Johnson, Spielman, Plastridge, Matterson and Nezvesky, 1949).

Boda and Cole (1954) and Boda (1956) attempted to prevent parturient hypocalcaemia by feeding a low calcium high phosphate diet to cows before calving, believing that by so doing they stimulated parathyroid activity. Conversely, Ender, Dishington and Helgebostad (1956; 1962) found that a high calcium low phosphate diet, particularly if it was alkaline, increased the incidence of milk fever. However, some of the diets used in these experiments were highly artificial and other dietary factors were probably altered as well. The practical application of the method of Boda and Cole is limited by the difficulty of producing a satisfactory precalving ration with a low calcium content.

Vitamin D was suggested by Greig in 1930 for the prevention of milk fever but it was not until 1955 that the technique became a practical proposition (Hibbs and Pounden). The vitamin D is administered in massive doses for a short time before calving, either orally or parenterally, and the efficacy of the method has been confirmed on a number of occasions (Weighton, 1958; Jonsson, 1958; Seekles, Reitsma and De Man, 1958; Dell and Poulton, 1958; Jonsson, 1960a; Hibbs and Conrad, 1960). The difficulty with oral administration is in pre-judging the date of parturition, since the vitamin must be given for not less than three days and not more than seven days immediately prior to parturition, otherwise toxicity may develop. The beneficial effect is lost if more than one day elapses between the end of the treatment and

parturition. Hibbs and Conrad (1960) attempted to protect 164 cows from milk fever; of these, only 113 satisfied the time conditions and in this group the method was eighty per cent effective. The vitamin D stimulates the absorption of calcium from the digestive tract of cattle and produces a positive calcium balance, but some of the calcium is apparently deposited in the walls of the heart and larger arteries (Swan, 1952; Conrad, Hansard and Hibbs, 1956; Greig, 1963).

More recently, attention has turned to hormone therapy as a prevention for milk fever. Prolan and progesterone are both reported to give satisfactory protection against the disease (Derlogea, 1956; Gerola, 1962) but variable results have been reported for ACTH (Westermarck, 1959; Jonsgard, 1963). Parathyroid extract is apparently without effect in raising serum calcium levels or preventing milk fever (Jackson et al., 1962).

There is, therefore, a need for a method which can be adopted when calving is seen to be imminent and whose effect would persist for about four days. The maintenance of alimentary activity at calving might prove to be the ideal solution, but failing that the essential object is to increase the labile calcium reserve within the body rather than depress milk secretion. The simplest method to apply would be the injection of a calcium compound in such a manner that it would be readily mobilised when required, but which would remain relatively inert in normal tissue fluids. Soluble salts of calcium are unsuitable for this purpose since they are taken up by the circulation and eliminated fairly quickly. Among the less soluble compounds is secondary calcium

TABLE XIV

Variations in the properties of commercial samples of secondary calcium phosphate.

Sample No.	pH of Suspension	Solubility in water at 37°C (mg./100 ml.)		
		Calcium	I.Phosphate	Ca x I.P.
I	7.60	3.82	14.77	56.4
II	6.91	2.84	12.34	35.0
III	7.62	4.68	4.18	19.6
IV	7.89	4.30	23.00	98.9

phosphate ($CaHPO_4.2H_2O$) which was found to have a varying solubility in blood. This section describes observations on its solubility in normal and hypocalcaemic blood and also on its ability to control hypocalcaemia when injected into sheep prior to infusions of sodium oxalate.

Materials and Methods

In vitro experiments:- The secondary calcium phosphate was obtained from four commercial sources and Table XIV gives some of the properties of each of these consignments.

The solubility of calcium phosphate in blood was determined by comparing the concentrations of calcium and inorganic phosphate in the plasma of heparinised cattle blood before and after equilibration with the calcium phosphate. The blood samples were obtained from normal and hypocalcaemic cows and in some cases portions of the normal bloods were partially oxalated prior to equilibration with the calcium phosphate (0.3 mg. sodium oxalate per millilitre of blood). In each experiment 50 mg. of secondary calcium phosphate was added to 25 ml. blood at the appropriate temperature and inverted fifty times by hand. The samples were mixed again after fifteen minutes of incubation and once an hour in prolonged incubations.

In vivo experiments:- Sheep of either sex of approximately one hundred pounds live weight and between one and two years old were used for the oxalate infusions. More than one experiment was performed on most sheep and a period of at least a month elapsed between successive trials. Each sheep was given an intravenous injection of one gram of

TABLE XV

Solubility at 37°C of secondary calcium phosphate in blood taken from cows before treatment and after recovery from milk fever

(mg./100 ml. plasma)

Cow No.	Before Treatment						After Recovery					
	Serum			Serum + $CaHPO_4.2H_2O$*			Serum			Serum + $CaHPO_4.2H_2O$*		
	Calcium	I.Phos.	Ca x I.P.	Calcium	I.Phos.	Ca x I.P.	Calcium	I.Phos.	Ca x I.P.	Calcium	I.Phos.	Ca x I.P.
1	7.05	0.83	5.9	13.30	5.92	78.7	10.13	4.30	43.6	12.50	6.18	77.3
	5.65	2.43	13.7	12.55	7.08	88.9	14.25	5.23	74.5	14.30	6.33	90.5
2	3.95	1.40	5.5	9.80	6.79	66.5	8.25	5.81	47.9	9.20	7.54	69.4
3	4.40	1.58	7.0	11.50	6.92	79.6	6.95	5.75	40.0	9.35	7.75	72.5
4	5.65	2.50	14.1	11.85	7.20	85.3	9.45	5.92	55.9	10.55	7.14	75.3
	4.30	3.65	15.7	9.85	7.70	75.8						
5	5.13	1.63	8.4	12.05	6.42	77.4						
	5.45	3.41	18.6	11.00	6.88	75.7						
Mean	5.20	2.18	11.1	11.49	6.86	78.5	9.81	5.40	52.4	11.18	6.99	77.0

* 2 mg. $CaHPO_4.2H_2O$/ml. blood.

sodium oxalate per hundred pounds live weight per hour for a period of three hours. No premedication was given to the control groups, but the experimental sheep each received 21.5 g. secondary calcium phosphate, which contains 5 g. calcium, intraperitoneally as a suspension in 250 ml. water two days prior to the oxalate injection. The injections were made through the right paralumbar fossa with the aid of a six centimetre needle and a flutter valve apparatus.

All in vivo experiments were performed with secondary calcium phosphate sample number III, but the preparation of the injection varied in different experimental groups. In one the material was given freshly mixed with water, in one the compound was washed thoroughly with water, while in two groups the compound was suspended in water and boiled for fifteen minutes and in one of these the acid so produced was removed by washing.

Biochemical and statistical analyses were by the methods described in Section II.

Results

Table XV shows the solubility of dicalcium orthophosphate (sample I) in blood taken from cows before treatment for parturient hypocalcaemia and after recovery. Those cows with double entries in the Table relapsed after the first treatment. The serum calcium level rose by an average of 6.29 mg. per cent in the hypocalcaemic samples but by only 1.37 mg. per cent in the normal samples. Inorganic phosphate levels also rose more in the hypocalcaemic samples than in the normal samples and the total

TABLE XVI

Solubility at 37°C of secondary calcium phosphate in normal and oxalated bovine blood

(mg./100 ml. plasma)

$CaHPO_4$ Sample No.	Blood Sample No.	Serum			Serum + $CaHPO_4.2H_2O$*			Serum + Sodium Oxalate**			Serum + Sodium Oxalate** + $CaHPO_4.2H_2O$ *		
		Calcium	I.Phos.	Ca x I.P.	Calcium	I.Phos.	Ca x I.P.	Calcium	I.Phos.	Ca x I.P.	Calcium	I.Phos.	Ca x I.P.
	1	11.80	6.9	81.4	12.80	9.00	115.2	2.10	7.50	15.8	8.80	16.5	145.2
	2	10.60	8.07	85.5	11.65	8.67	101.0	2.40	7.71	18.5	7.70	14.0	107.8
	3	11.00			12.30			2.00			7.80		
I	4	10.50			12.35			1.80			7.80		
	5	10.85			12.80			1.10			7.50		
	6	10.75			11.75			1.40			5.60		
	7	10.50			11.40			2.70			7.50		
	8	8.50	5.05	42.9	11.00	7.02	77.2	1.50	4.90	7.35	5.95	12.02	71.5
	9	9.04	5.32	48.1	8.85	5.20	46.0	3.00	5.08	15.2	3.10	4.64	14.4
	10	8.40	4.32	36.3	8.35	4.40	36.7	2.89	4.68	13.5	2.60	4.64	12.1
II	11	10.05	6.20	62.3	9.53	5.92	56.4	2.43	5.84	14.2	1.95	6.20	12.1
	12	9.51	5.20	49.5	9.14	5.50	50.3	2.33	5.40	12.6	1.90	5.06	9.6
	13							2.05	6.21	12.7	2.29	4.81	11.0
	9	9.04	5.32	48.1	10.05	6.41	64.4	3.00	5.08	15.2	7.55	9.00	68.0
III	10	8.40	4.32	36.3	10.88	6.28	68.3	2.89	4.68	13.5	8.05	9.48	76.3
	14	10.20	5.07	51.7	10.05	5.78	58.1	3.13	4.68	14.6	7.20	10.00	72.0
IV	13							2.05	6.21	12.7	7.15	10.15	72.6

* 2 mg. $CaHPO_4$ / ml. blood.

** 0.3 mg. sodium oxalate / ml. blood.

TABLE XVII

The effect of temperature on the solubility of dicalcium orthophosphate in plasma.

mg.Ca./100 ml.

	Incubation		
	Time min.	Temperature 12°C	37°C
Serum	0	10.50	10.50
Serum + $CaHPO_4.2H_2O$ *	0	11.70	12.35
	15	12.05	12.25
Serum + sodium oxalate **	0	1.80	1.80
Serum + sodium oxalate **	0	7.30	7.80
+ $CaHPO_4.2H_2O$ *	15	7.60	7.65

* 2 mg. $CaHPO_4.2H_2O$ / ml. blood.

** 0.3 mg. sodium oxalate / ml. blood.

TABLE XVIII

The effect of time of incubation at 37°C on the solubility of dicalcium orthophosphate in plasma.

mg.Ca./100 ml.

	Time of Incubation				
	Blood Sample 1		Blood Sample 2		
	0 mins.	15 mins.	15 mins.	2 hrs.	6 hrs.
Serum	10.50	10.50	11.00	11.05	10.70
Serum + $CaHPO_4.2H_2O$ *	12.35	12.25	12.30	11.95	12.30
Serum + sodium oxalate **	1.80	1.80	2.00	1.65	1.70
Serum + sodium oxalate ** + $CaHPO_4.2H_2O$ *	7.80	7.65	7.80	7.45	7.15

* 2 mg. $CaHPO_4.2H_2O$ / ml. blood.

** 0.3 mg. sodium oxalate / ml. blood.

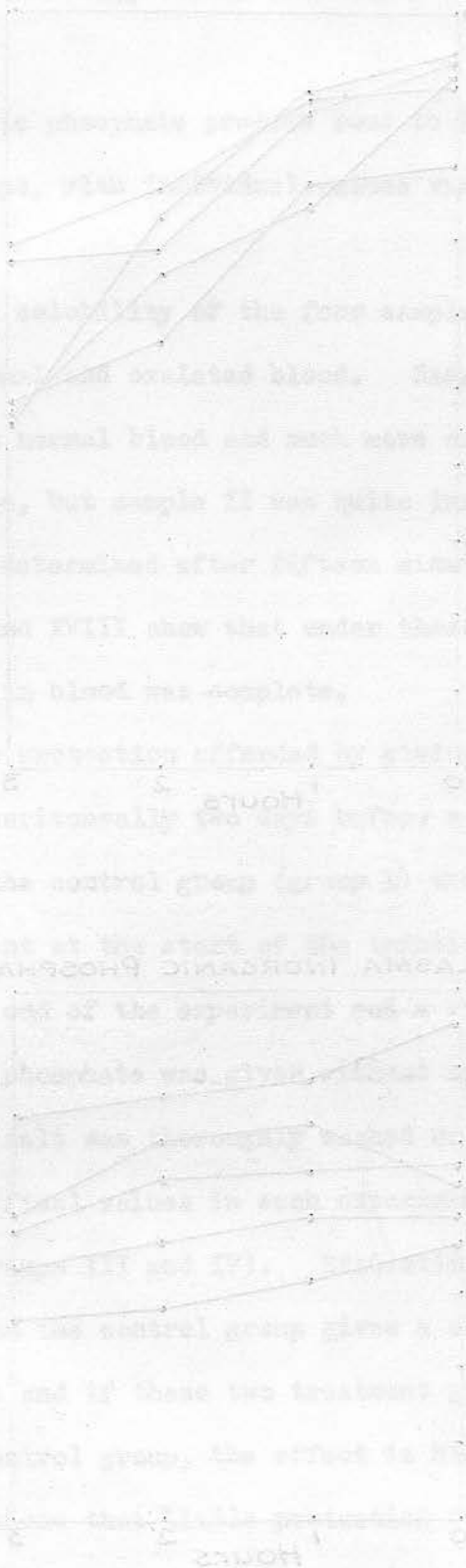
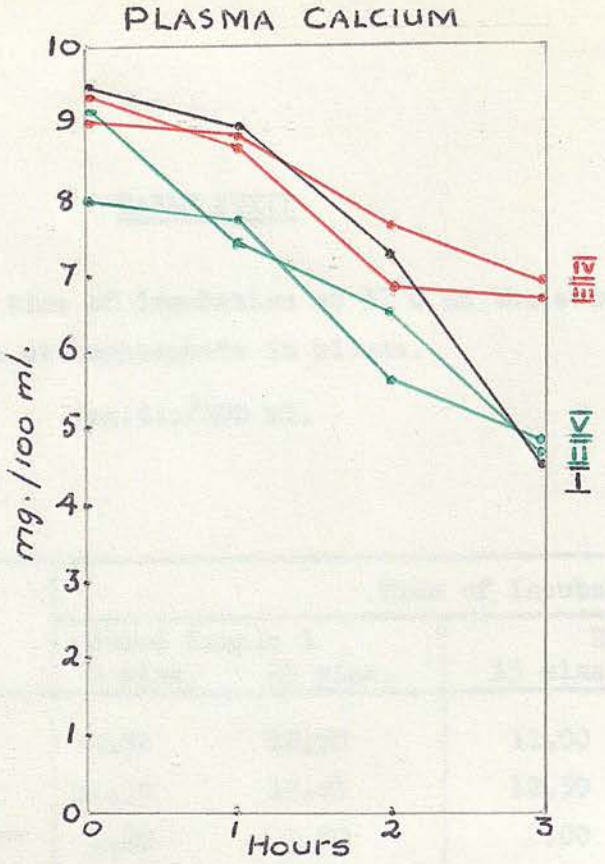
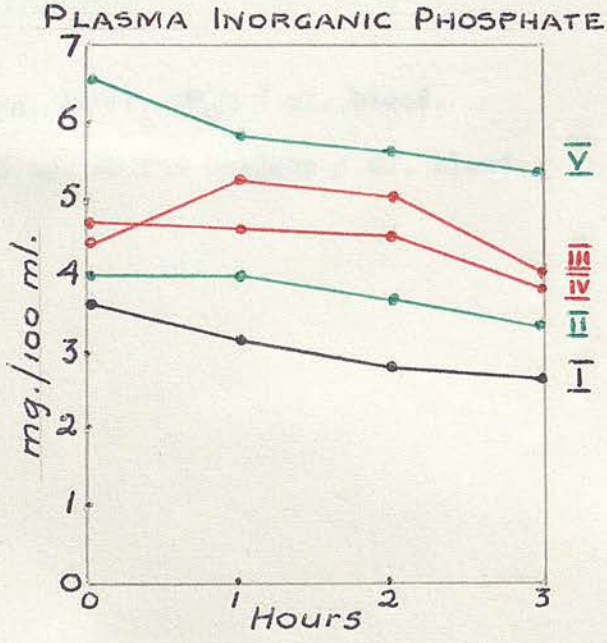


FIGURE 40 Plasma calcium and inorganic phosphate in sheep injected with sodium oxalate.





I Control Group — 4 animals
II $CaHPO_4 \cdot 2H_2O$, not washed, not boiled — 2 animals
III $CaHPO_4 \cdot 2H_2O$, washed thoroughly, not boiled — 2 animals
IV $CaHPO_4 \cdot 2H_2O$, boiled 15 mins. not washed — 2 animals
V $CaHPO_4 \cdot 2H_2O$, boiled 15 mins. and washed — 2 animals

From Tables A44 and A45.

plasma calcium x inorganic phosphate product rose to 78.5 and 77.0 mg. per cent in the two groups, with individual values ranging from 66.5 to 90.5

Table XVI shows the solubility of the four samples of secondary calcium phosphate in normal and oxalated blood. Samples I, III and IV were slightly soluble in normal blood and much more soluble in blood containing sodium oxalate, but sample II was quite insoluble. All these solubilities were determined after fifteen minutes of incubation at 37°C. Tables XVII and XVIII show that under these conditions the dissolution of sample I in blood was complete.

Figure 40 shows the protection afforded by giving 21.5 g. secondary calcium phosphate intraperitoneally two days before a three hour oxalate infusion. In the control group (group I) the plasma calcium fell from 9.5 mg. per cent at the start of the infusion to a mean of 4.6 mg. per cent at the end of the experiment and a similar pattern was found where the calcium phosphate was given without special preparation (group II). Where the salt was thoroughly washed or boiled before injection, however, the final values in each experiment lay between 6.5 and 7.0 mg. per cent (groups III and IV). Statistical comparison of each of these groups with the control group gives a significant difference for the treatment effect and if these two treatment groups are combined and compared with the control group, the effect is highly significant (Table A46). Group V shows that little protection was offered by the salt after both boiling and washing.

No definite differences are shown in the same figure for plasma

inorganic phosphate concentrations. In most experiments the plasma level fell, but there is the possibility that in group III the level was raised at one and two hours after the start of the experiment. The most noticeable feature was the high initial level of plasma inorganic phosphate in group V.

Discussion

Shear and Kramer (1930) were possibly the first to realise that normal blood is undersaturated with respect to secondary calcium phosphate. In their experiments on serum taken from four lambs and a calf, all six months old, they found that the serum calcium level altered only slightly after equilibration but the inorganic phosphate concentration rose, so giving an increased Ca x P product. The use of this empirical product was first introduced by Howland and Kramer (1922) and although methods of calculating solubility products are now available, this simple product is adequate for this discussion.

The solubility of secondary calcium phosphate in blood from cows was much greater than found by Shear and Kramer for a calf and lambs, for in normal plasma the product rose from 52 to 77, (Table XV), due to small increases in both plasma calcium and inorganic phosphate concentrations. Where blood was obtained from cows which were naturally hypocalcaemic and hypophosphataemic, the increases in the plasma calcium and inorganic phosphate concentrations on equilibration with secondary calcium phosphate were much more pronounced. The serum calcium increased by over 6 mg. per cent to 11.5 mg. per cent

but the final Ca x P value was the same as for experiments with normal plasma. These experiments with secondary calcium phosphate, which is probably a natural salt of calcium in the blood, give no indication of any alteration in the binding of calcium in the blood of hypocalcaemic cows, as has been suggested by Carlstrom (1961).

Where the plasma was oxalated prior to equilibration with secondary calcium phosphate, the plasma calcium concentration was elevated by about six milligrams per cent, but only to values of less than nine milligrams per cent. The final solubility product was similar to that for the blood of normal cows, principally due to a greater increase in inorganic phosphate. This type of effect was shown by calcium phosphate samples I, III and IV; sample II produced no effect on the calcium or phosphate levels of blood with which it was mixed.

Secondary calcium phosphate therefore appears to be sparingly soluble in normal blood but more readily soluble in hypocalcaemic blood and would perhaps be a useful prophylactic if administered parenterally to animals. A number of subcutaneous, intramuscular and intraperitoneal injections were made into sheep, using sample I, and in no case were untoward sequelae encountered. The subcutaneous and intramuscular injections produced very little local reaction and over a period of days showed early evidence of encapsulation. Material injected intraperitoneally was well dispersed throughout the peritoneal cavity, provided large volumes of fluid were used, and the last remnants were observed in sheep killed three weeks later.

When samples II and III were injected intraperitoneally various

degrees of abdominal discomfiture were produced. This led to the testing of the various methods of preparation of the injection shown in Figure 40. The material, mixed with water and injected immediately, produced no undesirable effects but also no protection two days later to oxalate induced hypocalcaemia. When boiled, the secondary calcium phosphate decomposed and the pH of the suspension fell to 4.8 or thereabouts. If the boiled material was injected without removal of the acid it caused anorexia for about twelve hours, but by the second day it seemed to offer protection against the severe hypocalcaemia of the oxalate administration. The same protection was found where the calcium phosphate was washed several times with distilled water before injection, and this seemed to cause less abdominal discomfort. Boiling and washing the salt before administration produced marked distension of the abdomen and no evidence of protection against oxalate hypocalcaemia.

So far no preparation of secondary calcium phosphate has been found comparable to sample I when injected intraperitoneally and unfortunately the source of this sample is not known. It had been standing on the laboratory shelf for some years and bore no manufacturers label.

The administration of sodium oxalate to these sheep would remove about 0.3 g. calcium per hour which is a relatively greater loss than would be expected in milking cattle. The difference between the concentrations of plasma calcium in the control group and groups III and IV in Figure 40 during the last hour of the oxalate infusion represents a difference in the loss from the total circulation of about 50 mg. calcium, or of about 0.25 g. calcium from the extracellular pool.

Assuming a mobilisation of 0.5 g. calcium per hour would be required from an injection of calcium phosphate to protect cows from parturient hypocalcaemia and that this could be taken up by the blood at a rate of 4 mg. per 100 ml., a total flow of ten to fifteen litres of blood per hour in the vicinity of the injection would be necessary. Even in the sheep the portal blood flow greatly exceeds this amount and it may be that the total quantity of calcium phosphate given in some of these experiments was taken up by the circulation within the two days which elapsed between its injection and the challenge infusion of oxalate. This is most likely to be true of the unwashed and unheated secondary calcium phosphate. Where washing or heating was carried out, as in groups III, IV and V of Figure 40, some of the injection may have been converted to tricalcium orthophosphate or hydroxyapatite. This latter compound is of variable composition and its formation probably takes place by a number of steps over a period of weeks (Logan, 1940; Neuman and Neuman, 1958). Unlike secondary calcium phosphate which is sparingly soluble in normal blood, these materials will not dissolve until the chemical composition of the blood alters, and in the sheep in these three groups the fall in plasma calcium concentrations did not seem to be arrested until the Ca x P product fell to between 30 and 40. This product might have some significance since Holt (1925) found an empirical Ca x P product of under 40 in the serum of rachitic rats and over 40 in the sera from normal rats and rats recovering from rickets.

It would appear from these experiments that secondary calcium phosphate would be either too labile or too soluble to form a satisfactory

preventive material for hypocalcaemia in cattle. Heating or washing the sample prior to injection probably hydrolyses some of the compound and the resulting material might have a preventive value.

Summary

The solubility at 37°C of secondary calcium phosphate in normal, naturally hypocalcaemic, and oxalated cattle blood was determined. Solubility was slight in normal blood but greater in blood with low calcium and phosphate levels, so that the plasma calcium x inorganic phosphate products (mg. per cent) were normally raised to between 60 and 80. Variations were noted in the solubility of different commercial preparations of secondary calcium phosphate.

Sheep were injected intraperitoneally with 21.5 g. secondary calcium phosphate (equivalent to 5 g. calcium) suspended in 250 ml. water. Two days later the sheep were given an intravenous injection of one gram sodium oxalate per 100 lbs. live weight per hour for three hours. At the end of the injection the plasma calcium of sheep injected with secondary calcium phosphate, which had been washed with water several times or boiled before injection, was two milligrams per cent higher than in the control animals, but the injection of the salt without washing or boiling, or after prolonged boiling and washing, was ineffective in controlling oxalate induced hypocalcaemia.

SECTION VI

THE ESTIMATION OF MINERAL ABSORPTION IN THE CONSCIOUS SHEEP

The need for continuous absorption of calcium from the digestive tract of cows was discussed in Sections II, III and IV and evidence was presented that absorption may be interrupted in cows at calving. Conventional balance techniques do not permit a study of this problem on an hour to hour or even on a daily basis, but veno-arterial techniques have commonly been used to study changes in the absorption of organic nutrients in anaesthetised sheep (Annison and Lewis, 1959). The only comparable work on calcium and phosphate absorption is that of Stewart and Moodie (1956) who reported differences in the composition of blood obtained from the carotid artery and various veins draining the digestive tract, but this method is not suitable for use in the conscious animal.

It was therefore decided to develop a more suitable technique using sheep as the experimental animal during the developmental period. A method for collecting portal venous blood was given in Section I and the primary object of the studies reported in this Section was the determination of normal veno-arterial differences between the plasma calcium and inorganic phosphate contents of arterial and portal venous blood samples, taking into account any dilution of the blood by absorbed water. At the same time the opportunity was taken to study the absorption

of magnesium and citric acid since both have been credited with influencing calcium absorption.

Materials and Methods

Animals:- Nine female sheep, aged nine months to seven years, and seven castrated male sheep, nine months to two years of age, were used. Five of the sheep were Suffolks, the rest were Border Leicester or Border Leicester cross Suffolk. Each animal was prepared with a carotid loop (Schambye, 1951) and a portal vein catheter as described in Section I.

The animals were fed liberally on good quality ryegrass hay, which sometimes contained clover, and a daily supplement of oats and ewe nuts. The quantities of supplement varied depending on the preferences of each animal, but generally consisted of about six hundred grams of mineral enriched nuts per day containing 1.37 per cent calcium and 0.65 per cent magnesium, and four hundred grams of oats containing 0.08 per cent calcium and 0.12 per cent magnesium.

Sampling:- One hundred and seventeen samples were obtained prior to morning feeding from sixteen sheep, ranging from one to eighteen samples per sheep. Samples were drawn from the carotid artery with a 19 or 20 B.W.G. serum needle, the blood being allowed to flow freely into the sample bottles, and the portal vein samples were taken simultaneously by hypodermic syringe.

Analyses:- The analytical techniques were standardised in order to detect small veno-arterial differences and the arterial and venous samples forming a pair were analysed simultaneously. The haemoglobin, calcium

and magnesium estimations were carried out in triplicate and inorganic phosphate and citric acid estimations in duplicate. All estimations were commenced on the day of collection and the plasma inorganic phosphate estimation was completed within four hours of collection to avoid errors due to storage (Moodie, 1962).

Haemoglobin was estimated by the method of King and Gilchrist (1947) using a Hilger Uvispek photoelectric spectrophotometer H.700,304. Plasma calcium was estimated by the method described in Section I and plasma magnesium by a modification of the method of Denis (1922) as described by Moodie and Walker (1963). Plasma inorganic phosphate and citric acid were assayed by the methods of Fiske and Subbarow (1925) and McArdle (1955).

The accuracy of the plasma estimations was verified by adding standard solutions to samples; in all cases 98-100 per dent of the added materials were recovered. The standard deviations of estimations on samples of plasma with or without added standard solutions, replicated eight to forty-eight times, were 0.03 to 0.06 mg. per cent for calcium, 0.01 to 0.04 mg. per cent for magnesium, 0.03 to 0.04 mg. per cent for inorganic phosphate and 0.14 mg. per cent for citric acid. The coefficient of variation for the haemoglobin estimations was 0.35 per cent. Since the haemoglobin, calcium and magnesium estimations were carried out in triplicate, the standard error of estimate for any sample was the S.D. $x\sqrt{\frac{1}{3}}$ and the standard error of the veno-arterial difference was the S.D. $x\sqrt{\frac{2}{3}}$. Inorganic phosphate and citric acid estimations were carried out in duplicate and their veno-arterial differences would be estimated

TABLE XIX

Example of calculation of apparent and corrected V-A differences, haemoglobin ratio and percentage haemoglobin differences.

(All calcium values are mg./100 ml. plasma and haemoglobin values are Uvispek readings)

Venous plasma calcium	...	11.65
Arterial plasma calcium	...	11.70
Apparent V-A difference	...	- 0.05
Venous haemoglobin concentration	...	0.795
Arterial haemoglobin concentration	..	0.754
Haemoglobin ratio (arterial Hb/venous Hb)	...	1.054
Percentage haemoglobin difference (100(Hb ratio - 1))	...	5.4
Corrected venous plasma calcium	...	12.28
Corrected V-A difference	...	0.58

with a standard error equal to their standard deviation.

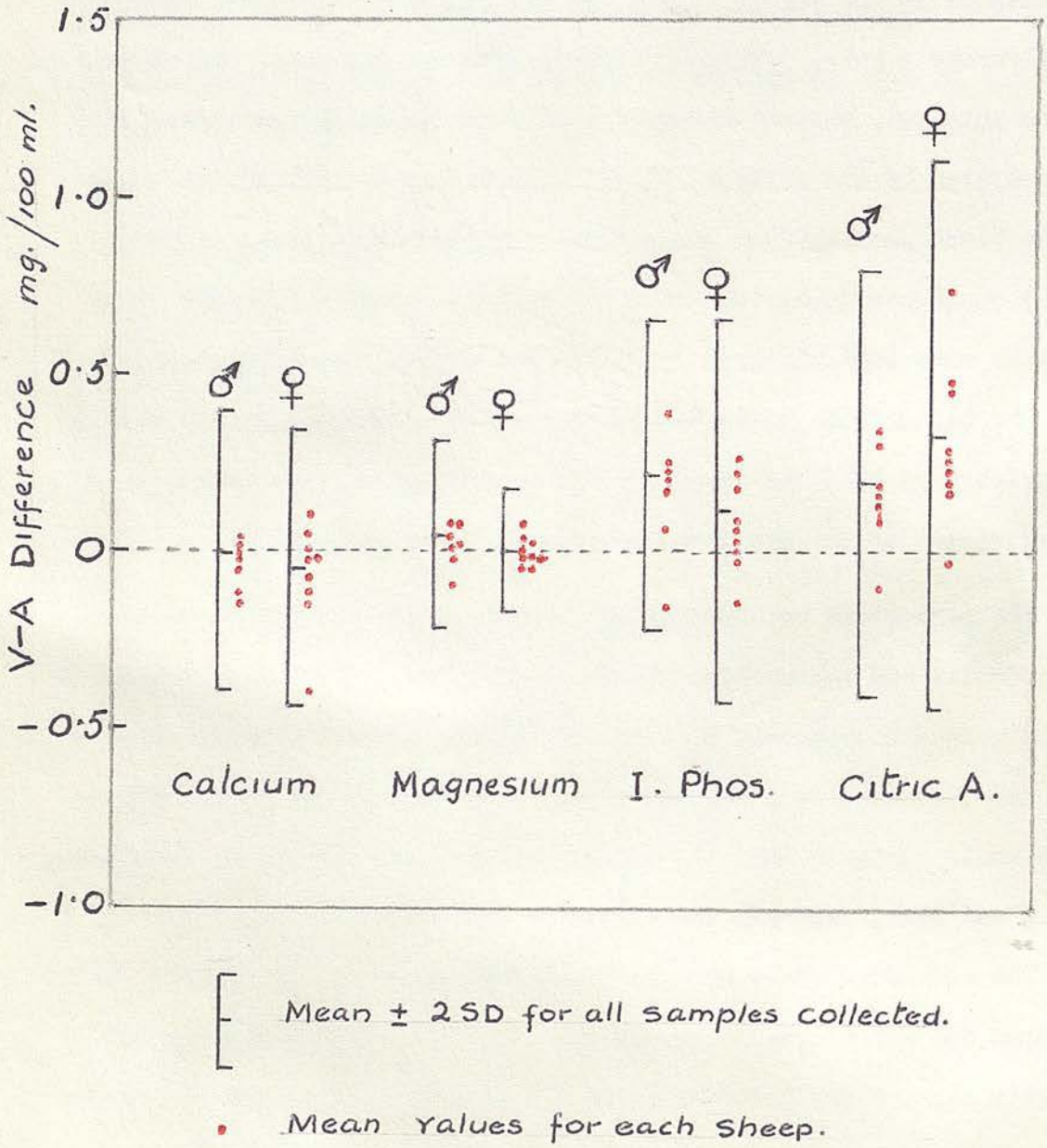
Calculation of Results:- The apparent veno-arterial (V-A) difference is the observed difference in the levels of the constituent in the portal venous and carotid arterial blood samples which formed a pair. This difference does not represent the absorption of the nutrient, because absorption of water drunk by the animal and secreted by its salivary glands must induce changes in the volume of the blood passing from the arteries to the portal vein. Dobson (1959) could not demonstrate the absorption of sodium from the rumen until he corrected his data for water absorption from that organ.

The haemoglobin ratio is the ratio of the arterial to the venous haemoglobin reading and represents an attempt to measure dilution or concentration of the blood passing through the splanchnic area.

The percentage haemoglobin difference is the difference between the arterial and venous haemoglobin readings expressed as a percentage of the venous haemoglobin reading and equals 100 (haemoglobin ratio - 1). This has been used as a measure of net water absorption. A negative value would indicate that the volume of the digestive juices (excluding saliva and bile) exceeded the volume of absorbed material.

The corrected veno-arterial (V-A) difference is the apparent data adjusted for water absorption and was determined by subtracting the concentration of the constituent in the arterial blood from the product of its concentration in the venous blood and the haemoglobin ratio.

FIGURE 41 : Means and distributions (mean ± 2 S.D.) of plasma calcium, magnesium, inorganic phosphate and citric acid apparent V-A differences for all samples collected from castrated male sheep and female sheep, and mean values for each sheep.



From Tables A49-A54.

TABLE XX

Standard deviations within sheep of apparent veno-arterial differences

mg./100 ml.

	Male	Female
Plasma Calcium	0.074	0.070
Plasma Magnesium	0.046	0.032*
Plasma Inorganic Phosphate	0.079	0.110**
Plasma Citric Acid	0.113	0.131

* and ** - Standard deviations differ significantly at 5 per cent and 1 per cent levels respectively.

From Table A53.

An example of the calculations is shown in Table XIX, a minus sign implying a loss of the constituent from the blood as it passes from the artery to the vein.

Statistical analyses were carried out according to Snedecor (1946).

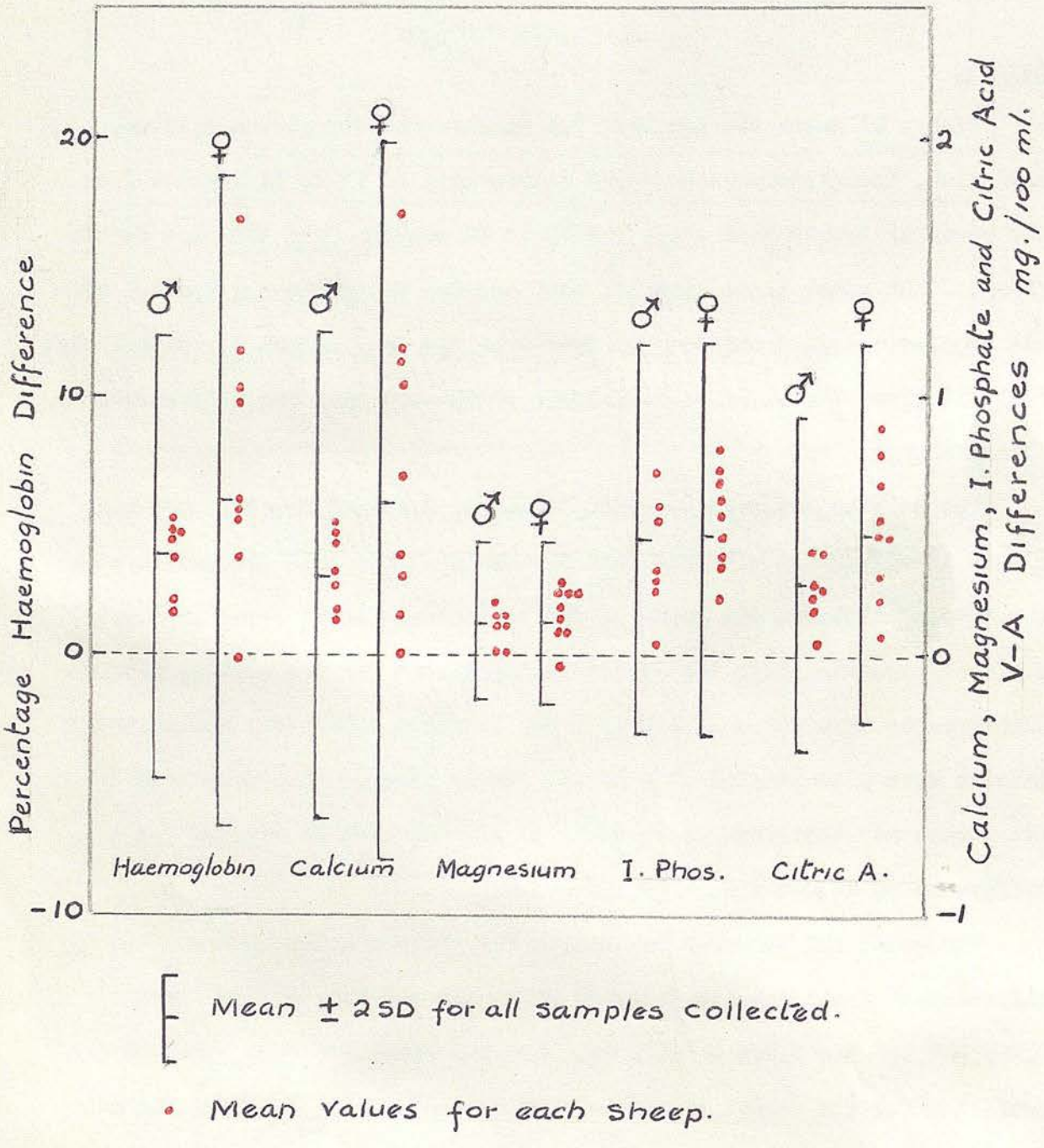
Results

Figure 41 shows the apparent V-A differences for plasma calcium, magnesium, inorganic phosphate and citric acid of 53 to 54 samples from the seven castrated male sheep and 59 to 60 samples from the nine female sheep. The black lines show the mean and the range (mean $\pm$ 2 S.D.) of all samples examined and the red dots show the mean values for each sheep. Table XX gives the standard deviations of the apparent V-A differences within sheep.

The calcium apparent V-A differences of male and female sheep had almost identical distributions and rather more than half the values were negative. Although the means of the individual female sheep (red dots) differed significantly, the distribution, except for one animal, was not much greater than for male sheep. The standard deviations within the animals were also similar in male and female sheep. The data from the two groups may therefore be combined to give an overall calcium V-A difference of -0.048 ± 0.195 mg. per cent.

The means and standard deviations for the magnesium apparent V-A differences of all samples obtained from male and female sheep were 0.045 ± 0.128 and 0.000 ± 0.084 mg. per cent respectively. The standard deviation for the female sheep was significantly lower than for the male

FIGURE 42 Means and distributions (mean ± 2 S.D.) of percentage haemoglobin difference, plasma calcium, magnesium, inorganic phosphate and citric acid corrected V-A differences for all samples collected from castrated male sheep and female sheep, and mean values for each sheep.



From Tables A49-A54.

TABLE XXI

Standard deviations within sheep of percentage haemoglobin differences and corrected veno-arterial differences.

	Male	Female
Percentage Haemoglobin Difference	1.6	1.8
Plasma Calcium (mg./100 ml.)	0.182	0.208
Plasma Magnesium (mg./100 ml.)	0.054	0.050
Plasma Inorganic Phosphate (mg./100 ml.)	0.133	0.144
Plasma Citric Acid (mg./100 ml.)	0.123	0.141

From Table A54.

sheep due to the greater uniformity of values within the female sheep (Table XX).

The deviations of the inorganic phosphate apparent V-A differences of the male and female sheep also differed significantly, but this time the values for the female sheep had the wider distribution. Both the mean values for individual female sheep and the deviations within the female sheep were greater than for the male sheep. The means and standard deviations of the values obtained from the male and female sheep were 0.218 $\pm$ 0.218 and 0.119 $\pm$ 0.272.

The apparent V-A difference for citric acid was 0.194 $\pm$ 0.298 in male sheep and 0.335 $\pm$ 0.387 in female sheep. These deviations were not statistically difference, but the means differed significantly at the five per cent level.

Figure 42 presents the percentage haemoglobin difference of male and female sheep and the corrected V-A differences for calcium, magnesium, inorganic phosphate and citric acid. As in Figure 41, the black lines give the means and ranges (2 x S.D.) of all samples analysed and the red dots show the means for each animal. Table XXI shows that the standard deviations within individual sheep were the same in both sexes.

The percentage haemoglobin differences of the female sheep had a higher mean and a greater range, particularly towards high positive values, than those of the male sheep. Since the variances differed significantly the means cannot be compared statistically, but it is clear that the greater variance of the female sheep was due to differences among the means of the individual animals (which were highly significant) and not to

TABLE XXII

Mean percentage haemoglobin differences and corrected veno-arterial differences of all samples obtained from castrated male sheep and female sheep.

V-A difference	Males			Females		
	n	Mean	S.D.	n	Mean	S.D.
Percentage haemoglobin difference	55	3.7	4.3	62	5.9	6.3**
Plasma calcium mg./100 ml.	54	0.301	0.475	60	0.587	0.694**
Plasma magnesium mg./100 ml.	54	0.127	0.150	60	0.118	0.158
Plasma inorganic phosphate mg./100 ml.	54	0.450	0.374	60	0.451	0.379
Plasma citric acid mg./100 ml.	53	0.272	0.321	59	0.465**	0.365

** Means or variances differ at the one per cent level of significance.

From Tables A51-A54.

differences within animals. Approximately three-quarters of the percentage haemoglobin differences were positive.

A comparison of the apparent and corrected V-A differences in Figures 41 and 42 shows that the use of the percentage haemoglobin difference as a correction factor for water absorption increased the mean value and the range of values for each plasma constituent and reduced the proportion of negative values. The use of this correction factor also made similar those apparent V-A differences which were not the same in male and female sheep, and made dissimilar those apparent differences which were the same in male and female sheep. Thus the plasma calcium corrected V-A differences of the female sheep had a wider distribution than those of the male sheep (due to differences among the means of the female sheep), but the plasma magnesium and inorganic phosphate corrected V-A differences were identical in male and female sheep. The citric acid data was not greatly influenced by the correction, except that the mean values were raised slightly. These corrected V-A differences are summarised in Table XXII.

Discussion

Attempts to estimate absorption from the gastrointestinal tract by examination of the blood depend on accurate measurement of the apparent V-A difference, the change in volume of the blood passing through the area under investigation and its rate of flow. The apparent V-A difference and change in volume of the blood only give information on the rate of uptake or loss of the nutrient per unit of blood and in the absence of

information on blood flow the rate of exchange between the tissues and the blood cannot be estimated. Therefore identical values for V-A difference and change in volume of the blood may represent quite different rates of exchange if the blood flow is different. The importance of this point has been disregarded in many acute experiments involving sampling from the veins of the digestive tract, where the abdominal contents were exposed either to handling or varying air temperatures.

Attempts to estimate the volume changes in the blood passing through the splanchnic bed by comparing the concentration of injected dyes in arterial and portal venous bloods were unsuccessful, since the dyes were continuously removed and any haemolysis in a sample upset the result. An attempt to overcome these problems was made by using haemoglobin as a natural marker, and the employment of the cyano-haemoglobin method of King and Gilchrist (1947) eliminated potential errors due to changes in the chemical form of the haemoglobin. Splenic contraction may influence the number of erythrocytes in the portal circulation, but it is unlikely to be important in trained sheep resting quietly. The effect of splenic contraction would be to reduce the percentage haemoglobin difference.

Assuming the total amount of haemoglobin in the blood does not change significantly as it passes through the splanchnic circulation, variations in haemoglobin concentration reflect changes in the volume of the blood, probably due to water. Thus the percentage haemoglobin difference probably represents the quantity of water gained or lost in the splanchnic region, in millilitres per 100 ml., and the haemoglobin ratio may be

used as a correction factor for determining the corrected V-A difference of whole blood constituents.

The use of the haemoglobin ratio as a correction factor for plasma constituents is open to theoretical objections, as this implies that the water absorbed by plasma and by cells is identical. Cells absorb water as they lose oxygen (Prankerd, 1960), and the cell volume of the blood passing through the splanchnic area probably increases by about five per cent and is independent of the haemoglobin ratio in sheep (Table A55). Nevertheless, the use of the ratio for plasma constituents gave corrected V-A values for plasma which, expressed in terms of whole blood, agreed closely with values obtained by whole blood analysis (Table A55). Ideally, all constituents should have been assayed on whole blood, but at the start of this series of experiments there was doubt about the accuracy of calcium and magnesium estimations on whole blood.

The percentage haemoglobin difference represents the absorption of water taken by the animal as food and drink and also the reabsorption of water from the salivary and biliary secretions, since these are not secreted by blood which passes through the portal vein on its return to the heart. The daily water intake of the animals in these experiments would be of the order of one litre per day and the total salivary and biliary flow was perhaps another 11.5 litres (Storry, 1961), giving a total of 12.5 litres. Assuming a portal blood flow of 1000 litres per day, that is, 700 ml. per minute (Fegler and Hill, 1958), the mean percentage haemoglobin difference would be 1.25 per cent.

Higher values than this were calculated for most animals in these experiments and the differences are too great to be accounted for by analytical error. True estimates of biliary and salivary secretion are, of course, very difficult to obtain and this may account for part of the difference, but another possibility is that the flow of blood through the portal system was slowed by the reaction to the catheter. A portal blood flow of 400 ml. per minute, for example, would almost double the expected percentage haemoglobin difference. Any excitement at the time of sampling might also slow the flow, but in most cases no excitement was apparent at the time of sampling. Anticipation of feeding might also have favoured water absorption by affecting the flow of ingesta along the bowel, as all the samples were taken immediately before morning feeding. This suggestion is supported by observations on one animal where samples were taken at different times twice daily for ten days. Walker and Moodie (unpublished data) also observed lowered percentage haemoglobin differences in sheep later in the day, where the average value for the control experiments lasting two hours was 2.2.

Tables XX and XXI show that large deviations in the haemoglobin differences and apparent and corrected V-A differences may occur in any series of samples taken from an animal, so a comparison of nutrient uptake by V-A difference must involve multiple sampling. For example, the percentage haemoglobin differences within female sheep, which influenced all the corrected V-A differences, had a standard deviation of 1.8, so ninety-five per cent of the values from an average female sheep with a mean percentage haemoglobin difference of 5.9 would be expected to

lie between 2.3 and 9.5 (mean $\pm$ 2 S.D.).

If three per cent of water is taken up by plasma traversing the splanchnic region and the concentration of a constituent in the arterial and venous plasmas are the same, then an uptake of the constituent will have occurred equal to three per cent of the concentration of the constituent in the arterial plasma. This concept forms the basis of the corrected V-A data which give more credible results than the apparent V-A data. For example, the calcium apparent V-A difference averaged -0.048 mg. per cent and if this represented the net exchange of calcium between the gut contents and the blood of these sheep it would imply a daily loss of 0.48 g. calcium per day, assuming a portal blood flow of 700 ml. per minute. This and the loss of calcium in the urine would mean that the animals were in negative calcium balance, despite a daily dietary intake of about nine or ten grams of calcium in these experiments which mostly involved young animals. Using the corrected data, however, the estimated daily absorption of calcium would be of the order of 3.0 and 5.9 grams per day for male and female sheep. This uptake represents absorption from the food and reabsorption of salivary and biliary calcium, but not of calcium from the other digestive secretions. Salivary and biliary calcium would account for approximately 0.3 grams per day (Storry, 1961), which gives absorptions of dietary calcium of about 2.7 and 5.6 grams, representing about 27 per cent and 56 per cent availability, in male and female sheep.

A similar calculation for magnesium, based on data from the same sources, gives a dietary magnesium of about four grams per day, total

salivary and biliary secretions of 0.15 g. per day and total absorption of 1.2 g. per day. The availability of the dietary magnesium in this case would be about twenty-five per cent. A calculation based on the apparent differences would give apparent availabilities of 0.3 and -0.15 g. per day for male and female sheep. This would not account for the normal urinary excretion of magnesium which, at a dietary intake of four grams per day, would probably be of the order of 0.4 to 0.8 g. per day (Field, McCallum and Butler, 1958).

Using the same sort of calculation, the corrected inorganic phosphate V-A difference indicates a daily absorption of 4.5 g. per day for both male and female sheep. Probably this largely represents reabsorption of salivary phosphate, since the parotid glands alone secrete perhaps two to six grams of phosphate per day (McDougall, 1948; Smith, Kleiber, Black and Baxter, 1955; Kay, 1960) and urinary excretion of phosphate in sheep is normally low (Wright, 1955; Crookshank and Robbins, 1962). The use of the apparent data would give an estimated daily absorption of only 2.2 g. and 1.2 g. phosphorus per day for male and female sheep.

These calculations of daily calcium, magnesium and inorganic phosphate absorptions were made solely to demonstrate that the corrected data give more credible values than the apparent data. All the samples on which the calculations were based were collected between 9 a.m. and 10 a.m., which probably accounts for the estimated calcium and magnesium absorptions, expressed on a daily basis, being slightly higher than expected. To obtain a true estimate of daily absorption by this method, it would almost certainly be necessary to make periodic collections of blood throughout

the day and night. The use of the results in this section must therefore be confined to comparative work, as, for example, a comparison of the absorption of nutrients under identical conditions.

The corrected data for the calcium V-A differences in Table XXII indicates that unless the mean portal blood flow of individual sheep varied greatly, the rate of absorption of this element differed in male and female sheep. There is no obvious explanation for this difference, since the animals were not selected in any way and no differences in feeding behaviour were noted. The position of the tip of the catheter may have varied among the animals causing samples of blood to be drawn from different streams within the portal vein (Garner and Singleton, 1953), but these differences should also have occurred at random.

The corrected V-A difference for any pair of samples (arterial and venous) will be influenced by the apparent V-A difference, the actual concentration of the substance in the portal blood and the percentage haemoglobin difference, as the following equation shows:-

$$\begin{aligned} \text{Corrected V-A difference} &= Vc - A \\ &= (Va \times \text{Hb ratio}) - A \\ &= Va \times \left(\frac{\%\ \text{Hb diff.}}{100} + 1\right) - A \\ &= \frac{Va \times \%\ \text{Hb diff.}}{100} + (Va - A) \end{aligned}$$

where Va = observed concentration in the portal venous sample,

Vc = corrected concentration in the portal venous sample,

A = concentration in the arterial sample,

TABLE XXIII

Correlations and regressions between the corrected veno-arterial differences for the various blood constituents and percentage haemoglobin differences.

Constituent	Sex	n	Relation between corrected V-A difference (y) and percentage haemoglobin difference (x)		
			Correlation	Regression	Standard Deviation from Regression $\sigma_{y.x.}$
Plasma Calcium	M	54	0.89	y=-0.055 + 0.097x	0.203
	F	60	0.95	y=-0.045 + 0.105x	0.210
Plasma Magnesium	M	54	0.54	y= 0.059 + 0.018x	0.127
	F	60	0.84	y=-0.010 + 0.021x	0.086
Plasma Inorganic Phosphate	M	54	0.78	y= 0.208 + 0.066x	0.238
	F	60	0.61	y= 0.231 + 0.036x	0.303
Plasma Citric Acid	M	53	0.26	y= 0.203 + 0.018x	0.313
	F	59	0.20	y= 0.385 + 0.012x	0.370

FIGURE 43 Regression of plasma calcium corrected V-A difference on percentage haemoglobin difference.

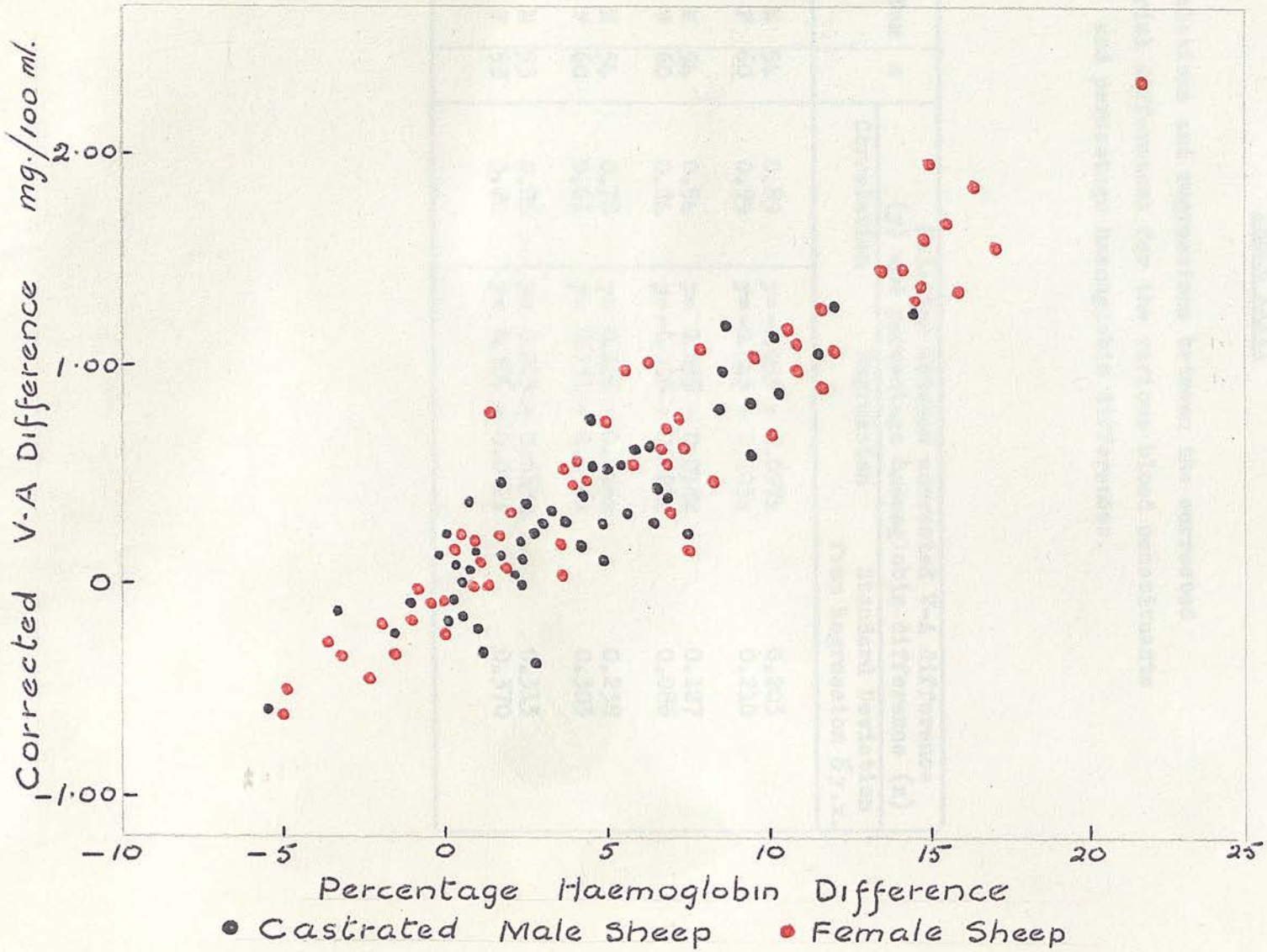


FIGURE 44 Regression of plasma magnesium corrected V-A difference on percentage haemoglobin difference.

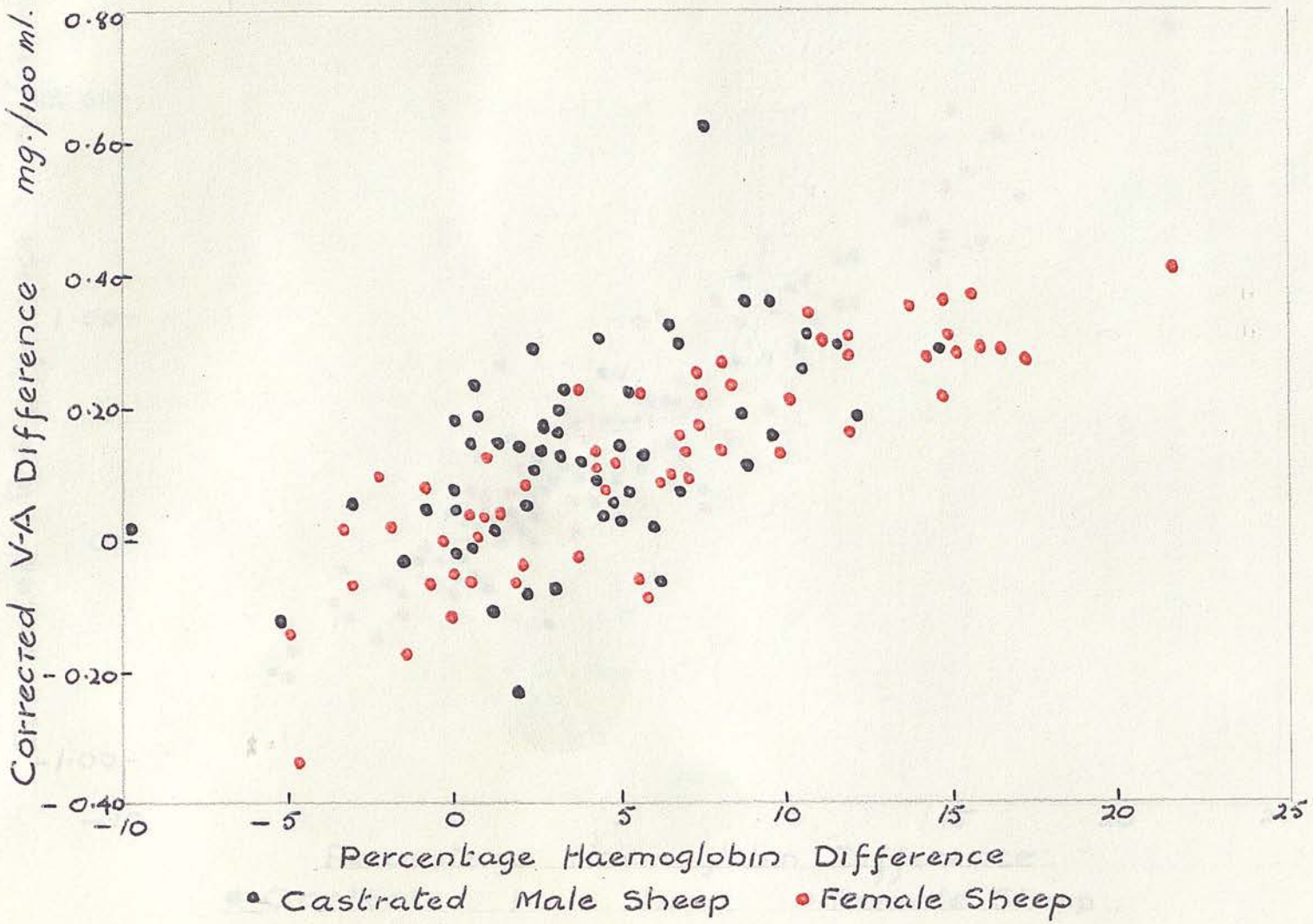


FIGURE 45 Regression of plasma inorganic phosphate corrected V-A difference on percentage haemoglobin difference.

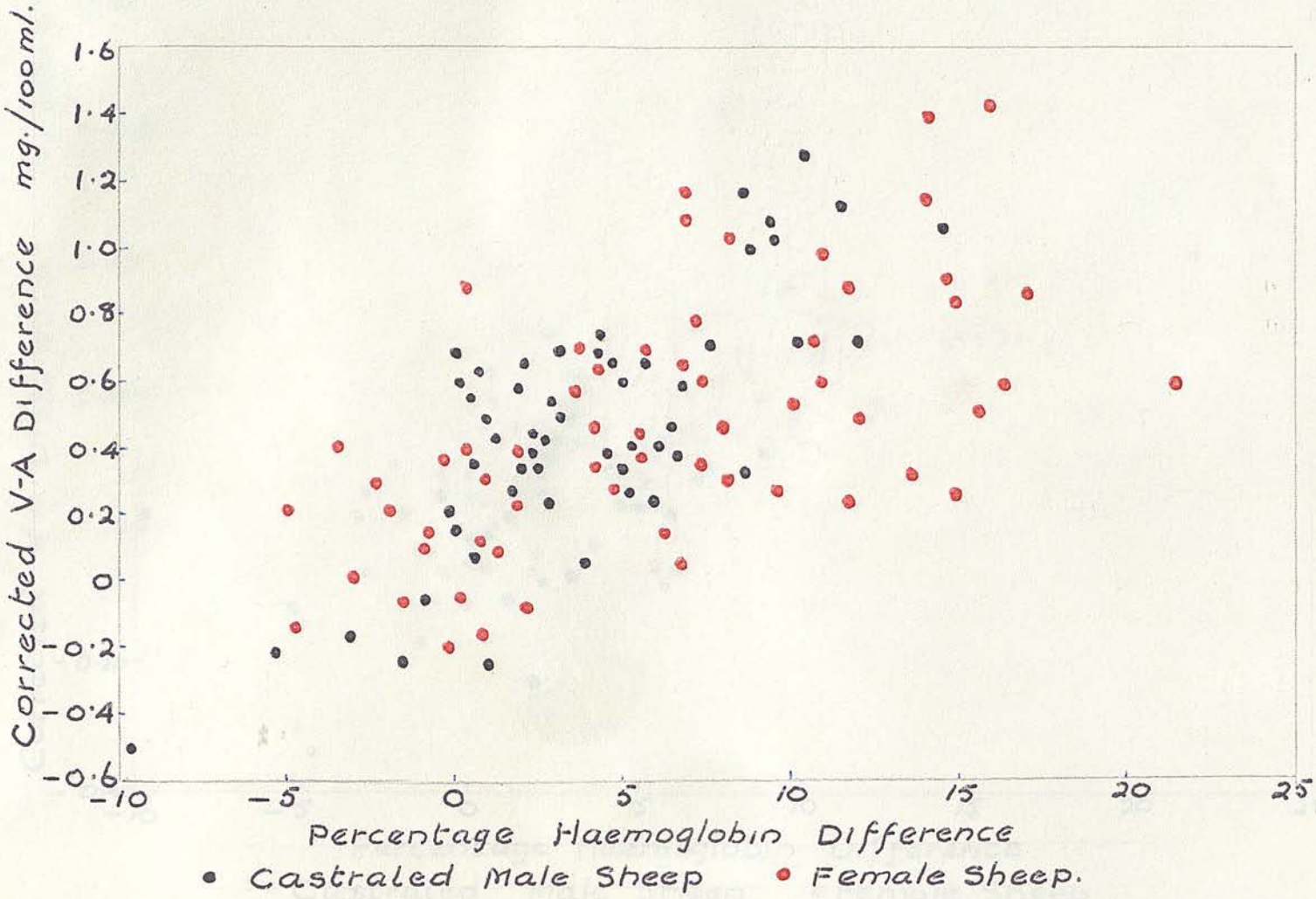
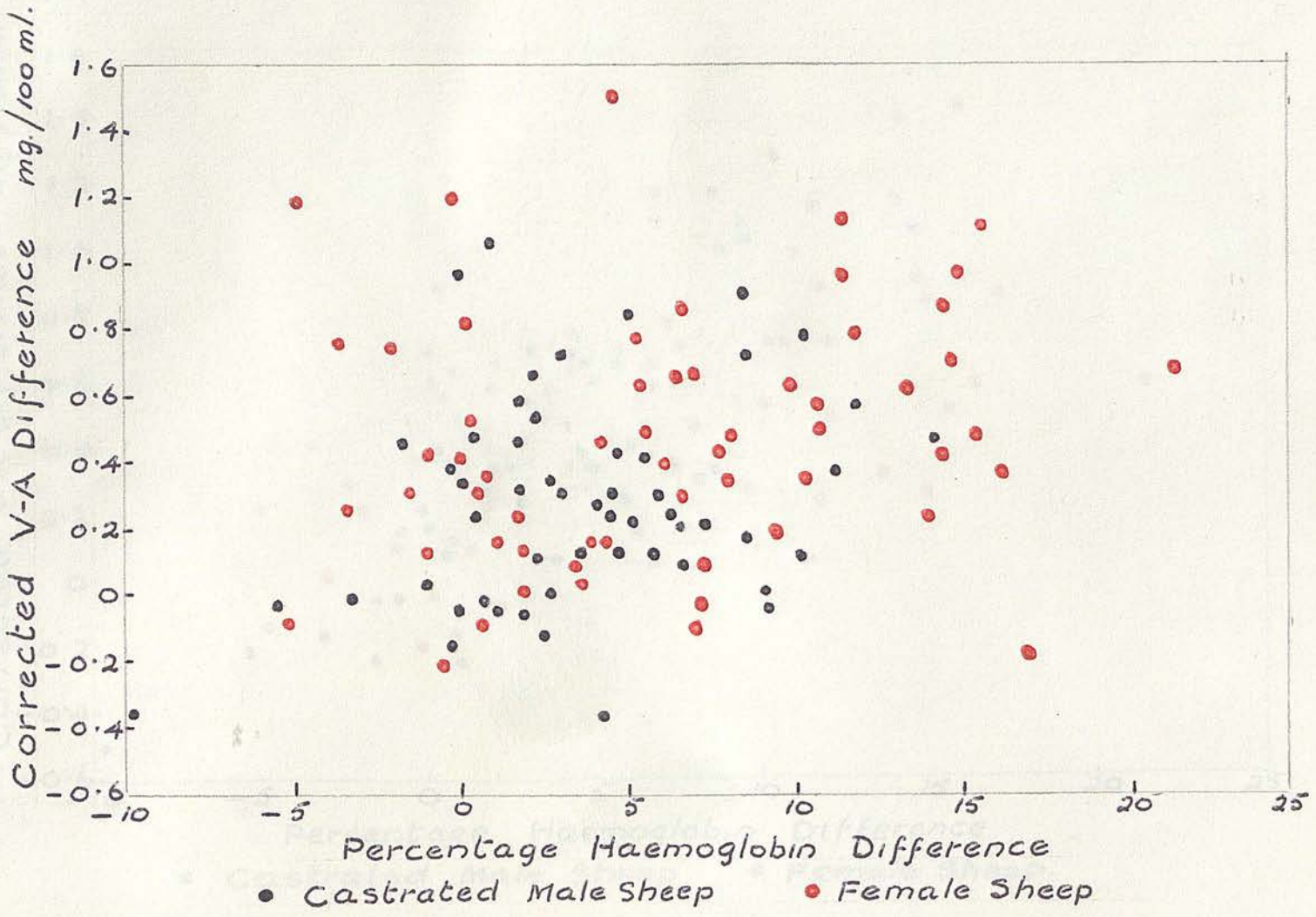


FIGURE 46 Regression of plasma citric acid corrected V-A difference on percentage haemoglobin difference.



and ∴ Va - A = apparent V-A difference

and Vc - A = corrected V-A difference.

Table XXIII shows the extent to which the percentage haemoglobin differences influenced the corrected V-A differences in this series of samples. Significant correlations were found for the calcium, magnesium and inorganic phosphate differences and where the correlations are high, as in the case of serum calcium, the constant a of the regression equation is the apparent V-A difference and the regression factor b is the portal venous plasma concentration divided by 100. The standard deviations from regression are similar to the standard deviations of the apparent V-A differences. These relationships can perhaps be observed much more clearly in Figures 43 to 46, where the corrected V-A differences for calcium, magnesium, inorganic phosphate and citric acid are plotted against the percentage haemoglobin differences.

Very good approximations can be obtained of the calcium corrected V-A differences by simply knowing the percentage haemoglobin difference, but this is progressively less true of magnesium, inorganic phosphate and citric acid.

Since all the corrected V-A differences are dependent to some extent on the percentage haemoglobin difference, there will obviously be a distinct tendency for the corrected V-A differences of calcium, magnesium, inorganic phosphate and citric acid to be positively correlated. Following the discussions of the previous sections, the association of calcium absorption with inorganic phosphate and citric acid absorptions are of particular interest, but the correlation between calcium and magnesium V-A

FIGURE 47 Correlation between plasma calcium and inorganic phosphate corrected V-A differences.

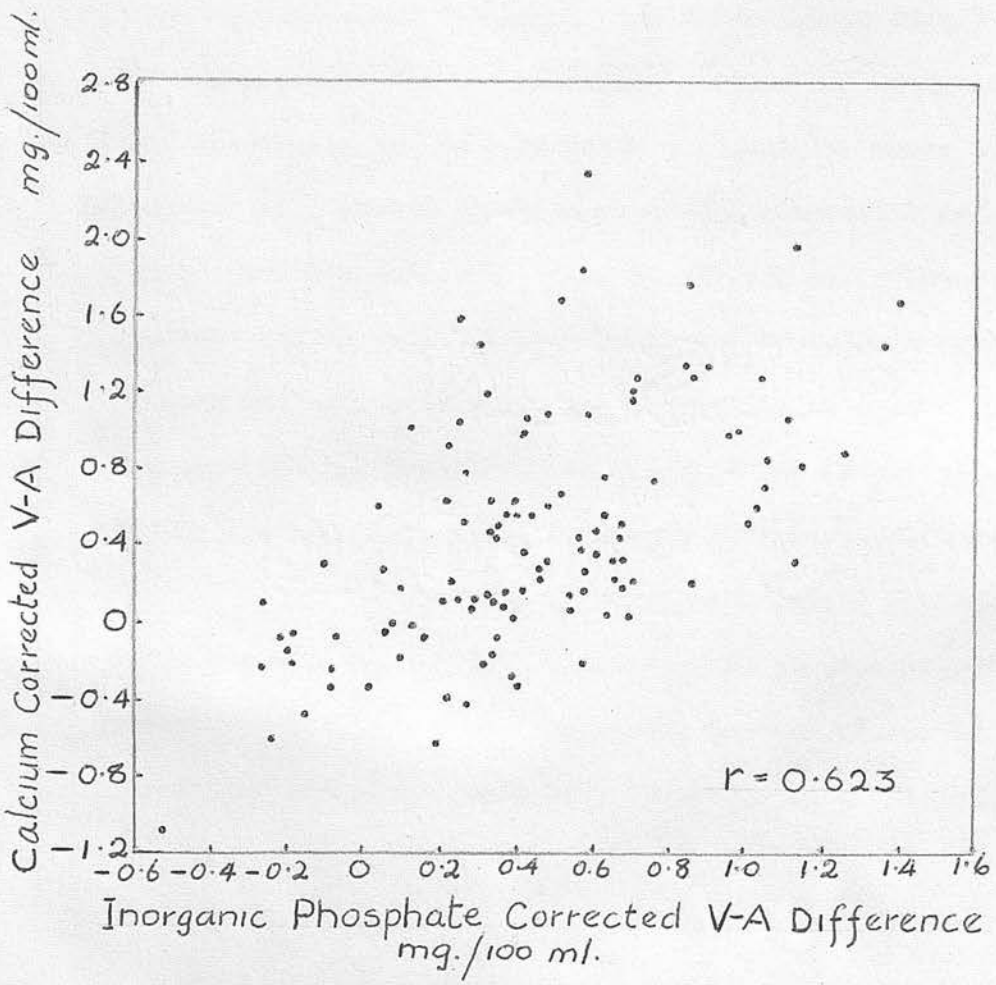
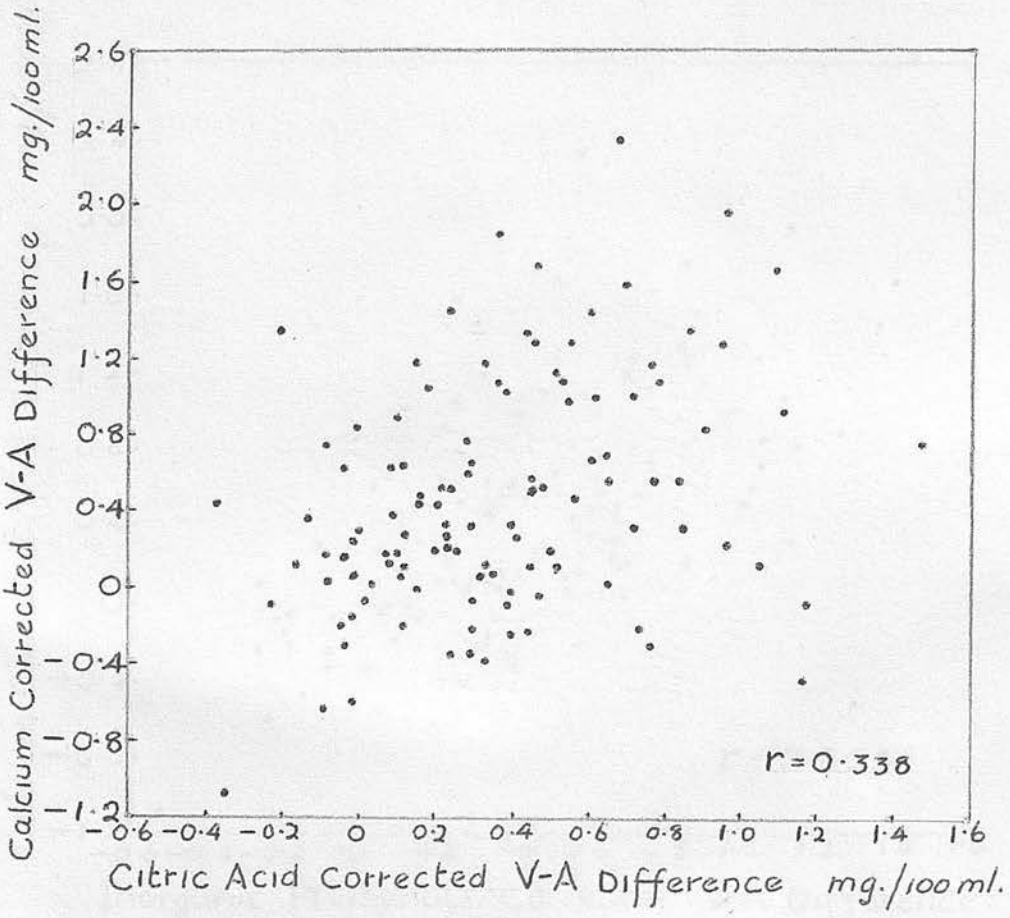


FIGURE 48 Correlation between plasma calcium and citric acid corrected V-A differences.



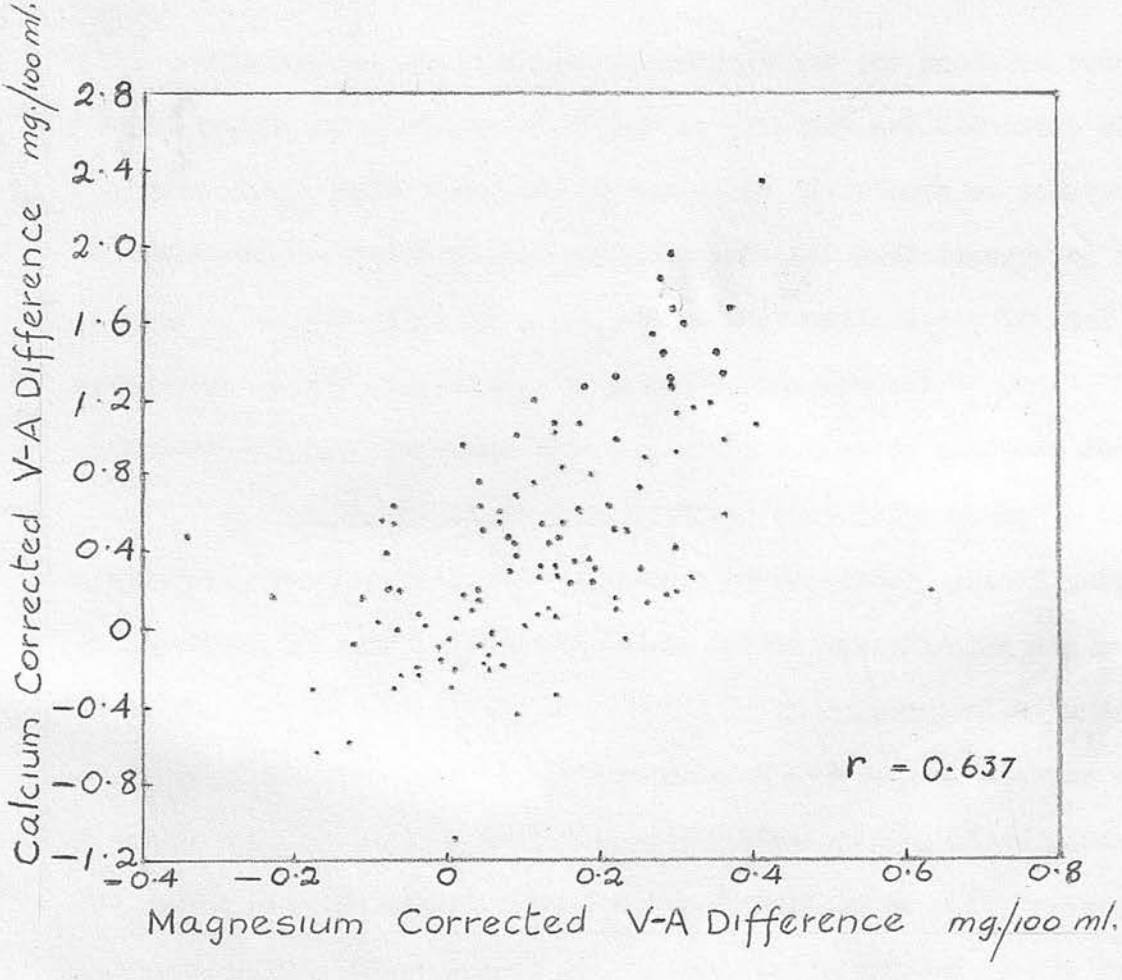
differences is also worth considering in view of the suggestion that these two substances have similar absorption pathways (Alcock and MacIntyre, 1962). These correlations are depicted in Figures 47 to 49.

Figure 47 shows the correlation between calcium and inorganic phosphate corrected V-A differences and indicates that the absorption of one may not be completely dependent on the absorption of the other. Although it appears that the absorption of both calcium and phosphate will be less if water absorption is impaired, as might happen in the event of stasis of the alimentary canal at calving, in these experiments it was not possible to relate changes in the corrected V-A differences of either of these substances to changes in their levels in the circulating blood. This result therefore adds little to our knowledge regarding the significance of low blood phosphate values in calving cows and cases of milk fever.

The correlation between calcium and citric acid corrected V-A differences, although statistically significant (Figure 48), indicates that the absorption of calcium from the intestine was not influenced to any great extent by citric acid metabolism in the alimentary tract. This conforms with the failure to establish any correlation between serum calcium and blood citric acid levels in the experiments reported in Sections III and IV.

The positive correlation between calcium and magnesium corrected V-A differences (Figure 49) might suggest that these two substances have a similar absorption mechanism, but the other results in this section do

FIGURE 49 Correlation between plasma calcium and magnesium corrected V-A differences.



not support this idea. The calcium apparent V-A differences for male and female sheep were similar but the magnesium apparent data differed; after correction for water absorption the calcium data for the two sexes differed but the magnesium data were similar. Calcium corrected V-A differences also appeared to be more closely associated with water absorption than did the magnesium V-A differences (Figures 43 and 44), a finding which agrees with the observation of Ross (1962) that the transport of magnesium across the wall of the rat intestine in vitro is not closely related to water transport.

It would appear, therefore, that under physiological conditions the absorption of calcium and magnesium in sheep need not be closely related, a view shared by Smith (1962) for calves, and the positive correlation between calcium and magnesium corrected V-A differences in Figure 49 probably reflects only the degree of correlation of each of these with water absorption. High calcium intakes in the diet, however, may impair the absorption of magnesium in the ruminant (Allcroft, 1961), but increased magnesium intake may improve calcium absorption, since Walker and Moodie (unpublished data) administered 0.5 g. magnesium as magnesium chloride into the jejunum of sheep and found evidence of absorption of water, calcium and magnesium. This was probably not a chloride effect, as the administration of 1.0 g. of calcium as calcium chloride produced a fall in the percentage haemoglobin difference, a temporary decrease in the magnesium corrected V-A difference and no evidence of an increase in the calcium corrected V-A difference. In laboratory animals under deficiency conditions there is evidence of antagonism in the absorption

of calcium, magnesium and phosphate, but how far the same is true of ruminants has not been determined (Schachter and Rosen, 1959; Toothill, 1963).

Summary

A method is described for investigating the absorption of nutrients in conscious sheep by comparing the composition of arterial and portal venous bloods. One hundred and seventeen pairs of samples were obtained from sixteen sheep (seven castrated male and nine female) and analysed for haemoglobin, plasma calcium, magnesium, inorganic phosphate and citric acid contents. Changes in the volume of the blood passing through the splanchnic area were estimated by comparing the haemoglobin concentrations in arterial and portal blood samples.

The veno-arterial differences for each constituent before and after correction for changes in the volume of the blood are presented and the necessity for making this correction is discussed. There is evidence of differences between male and female sheep and among individual female sheep in the rates of absorption of calcium, but the estimated absorptions of magnesium and inorganic phosphate were the same in both sexes. The citric acid corrected veno-arterial differences were greater in female than in male sheep.

The calcium corrected veno-arterial differences were closely related to water absorption, but the magnesium and inorganic phosphate differences were not influenced to the same degree by changes in haemoconcentration. Citric acid was absorbed independently of water. Calcium and citric acid

corrected V-A differences did not seem to be associated to any physiological extent. The relationship between the absorption of calcium and the absorption of magnesium and inorganic phosphate is discussed, and the possibility of stasis of the bowel interfering with both calcium and phosphate absorption was not excluded.

SECTION VII

THE EFFECT OF TRANSFUSING PLASMA FROM PARTURIENT COWS TO LAMBS

There are major changes in the hormonal balance of animals at parturition and these might lead, either directly or indirectly, to changes in the concentrations of calcium or phosphate in the plasma of cows at calving. In this section experiments are reported which were carried out in 1954-57 (before the possibility of hormone induced alimentary stasis at calving was appreciated) in an attempt to detect an active principle in the blood of calving cows which is not present in non-parturient cows and which has a depressent action on the plasma calcium and inorganic phosphate levels of ruminants.

Biological tests may be used to identify the presence of a hormone in blood, as, for example, the detection of follicle stimulating hormone in the serum of pregnant mares by mouse or rat inoculation (Cowie, 1948). Since parathyroid hormone does not seem to have the same effect on the plasma calcium and inorganic phosphate of ruminant and monogastric animals, other hormones might also show species differences, and therefore small lambs showing evidence of rumen development were used in these experiments in preference to other laboratory animals. The lambs were injected with plasma drawn from cows before, during and after calving and the changes in the plasma composition of the lambs were observed at frequent intervals for the succeeding forty-eight hours. Some of the experiments were carried out in Australia and the remainder in Scotland.

Materials and Methods

Animals:- The donor animals in the Australian experiments were mature Jersey cows which were never housed and were fed on native pastures supplemented with lucerne hay and a concentrate supplement. In the Scottish experiments pure-bred Ayrshire cows were used which were housed in winter and fed ryegrass hay containing some clover, root crops and a balanced production ration. The Queensland cows suckled their calves for three days but the Scottish cows were not allowed to suckle.

Merino lambs, of either sex, weighing 25 - 40 lbs. and about three months old were injected with plasma from the donor cows in the Queensland experiments. Some of the lambs were purchased in the open market but most were obtained from a neighbouring research organisation. In Scotland a variety of lambs were used, including Blackface, Cheviot, Greyface and Halfbred most of which were bought in the open market. These lambs weighed from 30 - 84 lbs. since the lambing season was much shorter than in Australia where small lambs were obtainable at any time.

Sampling:- For transfusion purposes one to two litres of cow blood was collected in heparinised bottles through a large cannula inserted into the jugular vein. This blood was immediately refrigerated and the plasma separated within twenty-four hours by centrifugation. In four of the Queensland experiments small samples were taken more frequently to study the normal changes in serum calcium and plasma

inorganic phosphate in calving cows.

Minimum quantities of blood were drawn from the lambs by means of a hypodermic syringe and needle inserted into the jugular vein. The blood was heparinised and the calcium and inorganic phosphate concentrations were estimated on the plasma. Samples were collected before the transfusion and at five to ten minutes, one, two, four, six, twelve, twenty-four, thirty-six and forty-eight hours after the transfusion. In the Queensland experiments an extra sample was taken at thirty minutes and in the Scottish experiments at eighteen hours after the transfusion.

Transfusion:- The plasma transfusions were made two to seven days after the collection of the plasma, which was stored continuously at $4^{o}C$. The dose varied between two and three millilitres per pound body weight, depending on the availability of plasma, and averaged 2.33 ml. and 2.12 ml. for the Australian and Scottish experiments respectively. The injections were made intravenously in about three minutes.

In Queensland transfusions were made with plasma collected from eight cows two to ten days before calving, and from seven of these within one hour of calving. The pre-calving samples served as controls. A transfusion was also made with plasma obtained from one of these cows twelve hours post-partum. In a further six experiments, lambs were infused with plasma from three milk fever cows, samples for transfusion being obtained before treatment and after recovery.

In Scotland the control experiments consisted of five transfusions

with samples collected three months before calving in one case and four to fourteen days after calving in the others. Six calving samples were collected between one hour before and two and a half hours after calving, and five post-partum samples between twenty-four and thirty-three hours after calving. Five experiments were repeated where the lambs were sampled only before and at twenty-four hours after the transfusion and a further seven samples obtained from non-parturient cows were used in this way.

Altogether, fifty lambs were injected with plasma, twenty-two in Queensland from twenty-two samples obtained from eleven cows and twenty-eight in Scotland from twenty-three samples obtained from thirteen cows. Particulars of the plasmas transfused (time of collection in relation to calving, and calcium and inorganic phosphate contents) and the lambs used in these experiments are shown in Tables A56, A57, A58 and A66.

Portions of four of the Queensland bovine control plasmas and four parturient plasmas from the same cows, as well as portions of the control, parturient and post-parturient samples of one Scottish cow, were extracted with ethanol. From these eleven samples, twelve infusions were made into lambs (Table A71). The preparation of each infusion was as follows:-

Three volumes of 95 per cent ethanol were added to one volume of plasma while shaking. The mixture was centrifuged, the supernatant removed and the residue washed with one volume of ethanol. The ethanol extracts were combined and reduced under vacuum to less than the original

plasma volume, the evaporation being assisted by heating in a waterbath at 40°C. The reduction was continued until the concentrate no longer smelt strongly of alcohol, when the extract was diluted to the original volume with 0.9 per cent sodium chloride solution. The infusions into lambs were made at approximately the same dosage levels as for the corresponding plasma transfusions (Table A71).

Six more lambs were infused with standard phosphate solutions containing 3.0 or 6.0 mg. P. per cent at the dose rate of 2.5 ml. per pound body weight. The solutions were prepared by dissolving 5.775 g. disodium hydrogen phosphate and 2.191 g. potassium dihydrogen phosphate in one litre of normal saline. This solution had a pH of 6.9 and was diluted to give the required phosphate concentration (Tables A69 and A70).

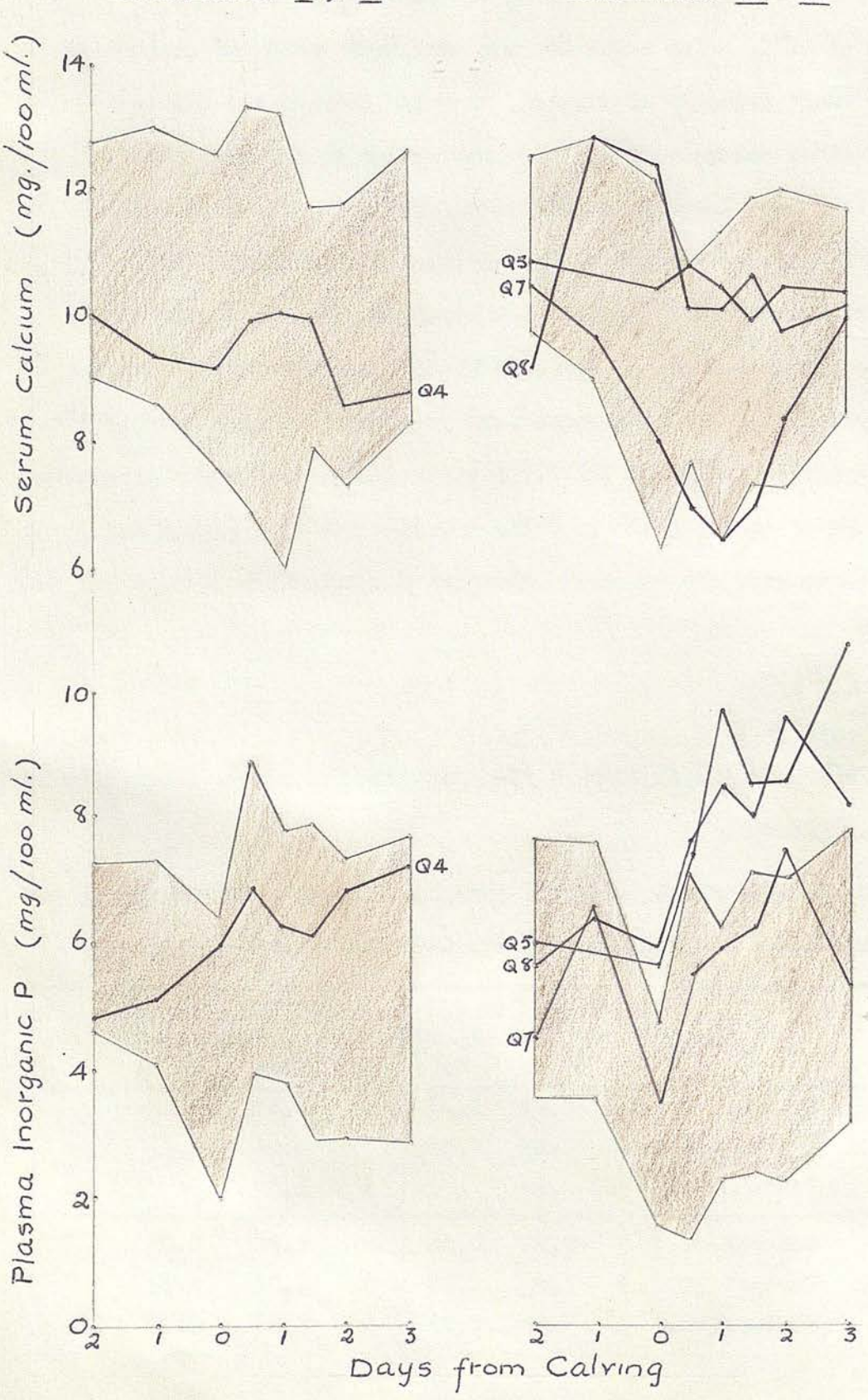
Results

(a) The concentration of calcium and inorganic phosphate in the plasma of donor cows

The mean calcium and inorganic phosphate contents of the normal cow plasmas which were used for transfusion into lambs were as follows:

		Plasma Calcium mg./100 ml.			Plasma inorganic phosphate mg./100 ml.		
		n	Mean	S.D.	n	Mean	S.D.
Queensland experiments	Control	8	10.79	0.77	8	5.37	1.19
	Parturient	6	10.19	1.49	7	4.66	1.17
	Post-partum	1	9.75		1	5.38	
Scottish experiments	Control	7	9.91	0.96	12	4.98	1.25
	Parturient	6	7.64	1.76	6	2.88	1.51
	Post-partum	5	7.49	2.27	5	4.68	1.13

FIGURE 50 Comparison between serum calcium and plasma inorganic phosphate levels of four Queensland cows at calving and values obtained for Scottish cows by Moodie, Marr and Robertson, 1955.



From Table A59.

These values were similar to those observed by Moodie, Marr and Robertson (1955) for calving cows in Scotland, except that the inorganic phosphate values for Queensland cows at calving were slightly higher than expected. This was partly due to three of the seven cows showing an increase in inorganic phosphate values at calving compared with their precalving values (Table A56), which is in contrast to earlier Scottish observations where all twenty-seven cows observed closely at calving showed a fall in plasma phosphate values (Moodie, 1952). This difference in the behaviour of the plasma phosphate concentrations of Queensland and Scottish cows is highly significant ($X^2 = 7.92$).

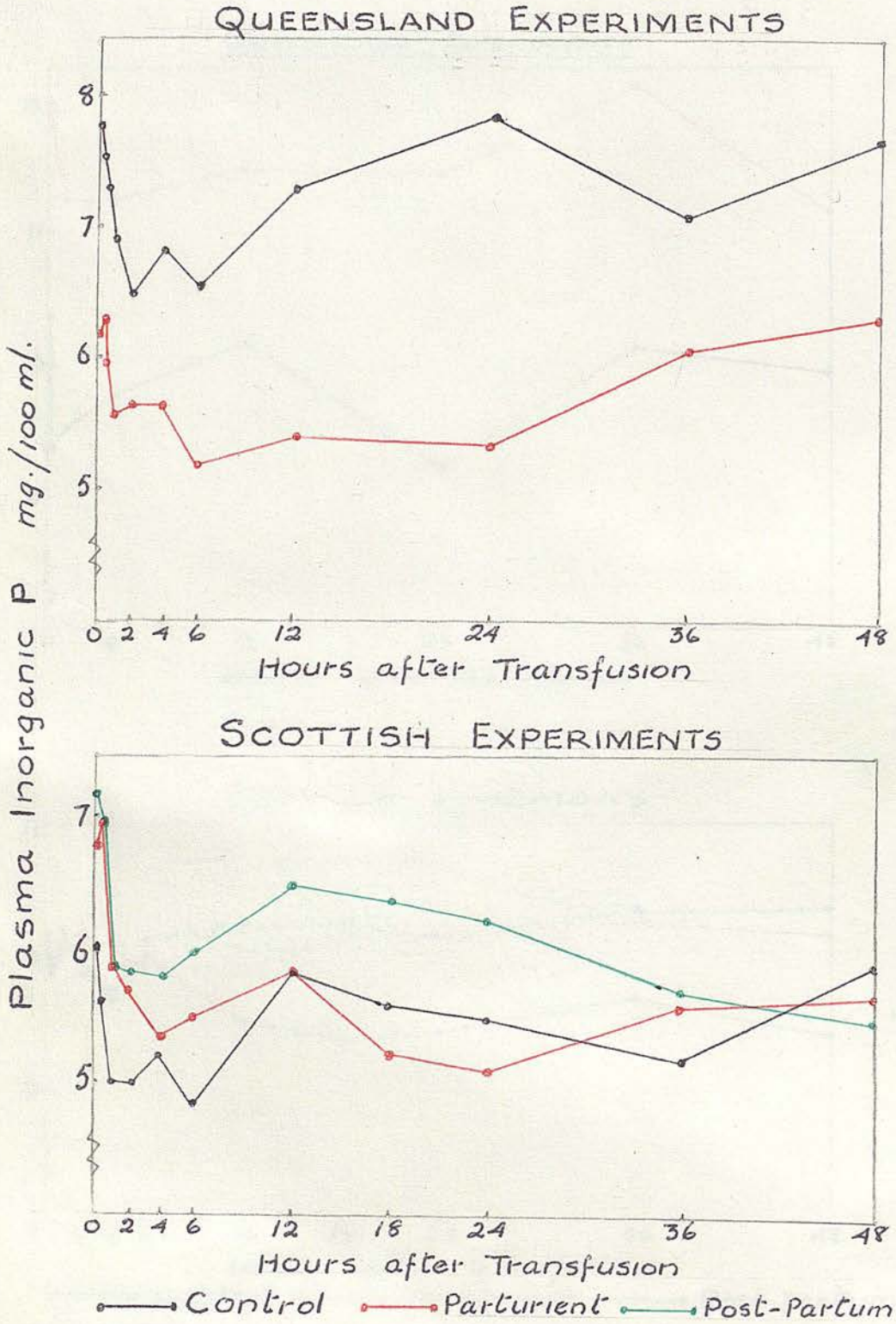
More frequent observations were made on the concentrations of calcium and inorganic phosphate in the plasma of four calving cows in Queensland. The results are shown in Figure 50 where the shaded areas show the range of values previously found for Scottish cows (95 per cent fiducial limits abstracted from Moodie, Marr and Robertson, 1955). No differences were observed between the calcium values for the Queensland and Scottish cows, but the pattern of the changes in inorganic phosphate tended to differ in the two countries. In Queensland the plasma phosphate concentration in the young cow rose at calving whilst in two of the old cows the concentrations increased for two or three days after calving to values much higher than normally expected for Scottish cows of the same age group.

The concentrations of calcium and inorganic phosphate in the plasma of the three milk fever cows before and after treatment were within the expected ranges (Table A66).

FIGURE 51 Plasma calcium concentrations in lambs following the intravenous injection of parturient and non-parturient bovine plasma at the rate of 2-3 ml. per lb. body weight.

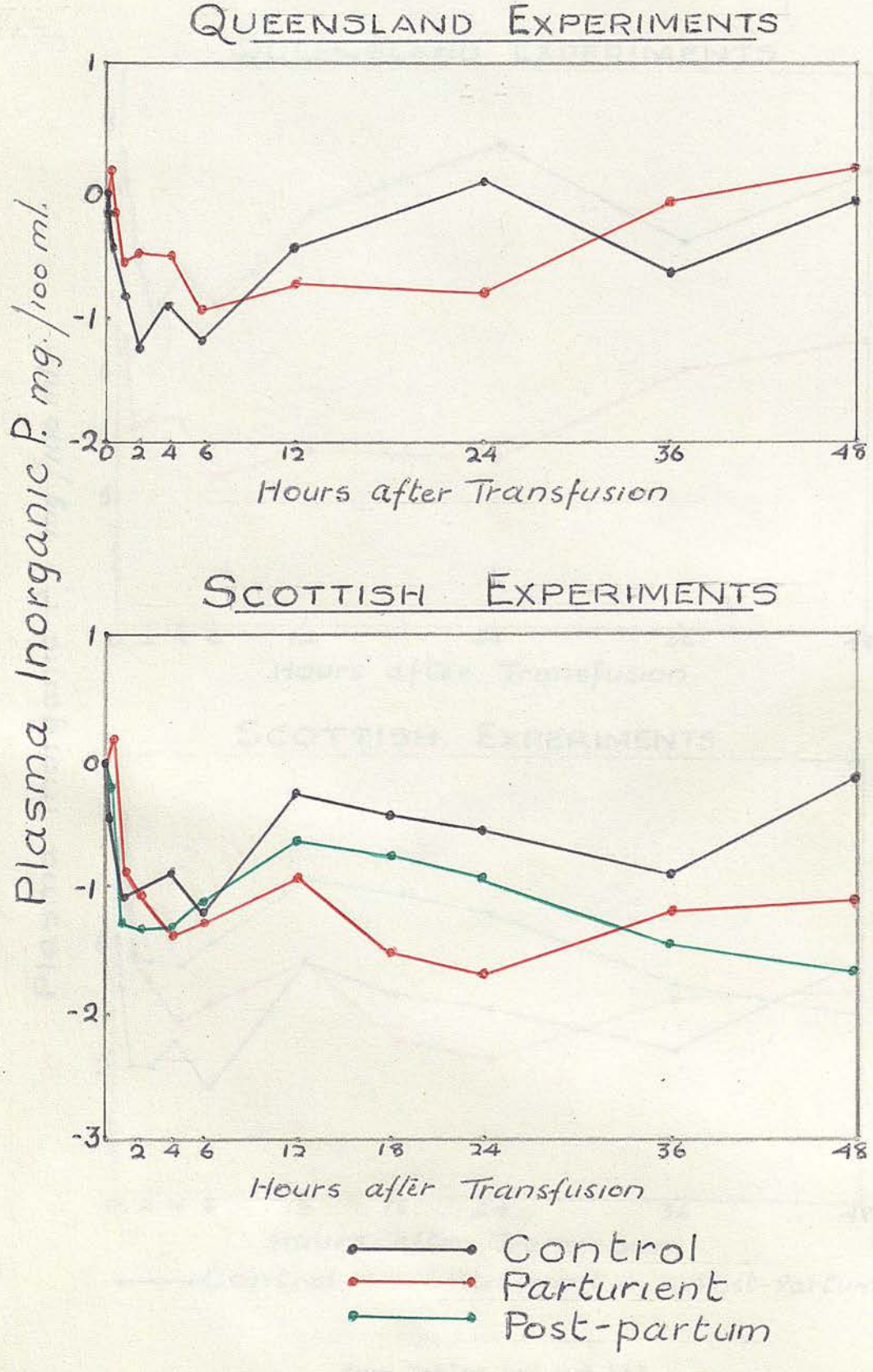
From Tables A60 and A61.

FIGURE 52 Plasma inorganic phosphate concentrations in lambs following the intravenous injection of parturient and non-parturient bovine plasma at the rate of 2-3 ml. per lb. body weight.



From Tables A62 and A63.

FIGURE 53 Changes in plasma inorganic phosphate concentrations in lambs following the intravenous injection of parturient and non-parturient bovine plasma at the rate of 2-3 ml. per lb. body weight.



From Tables A64 and A65.

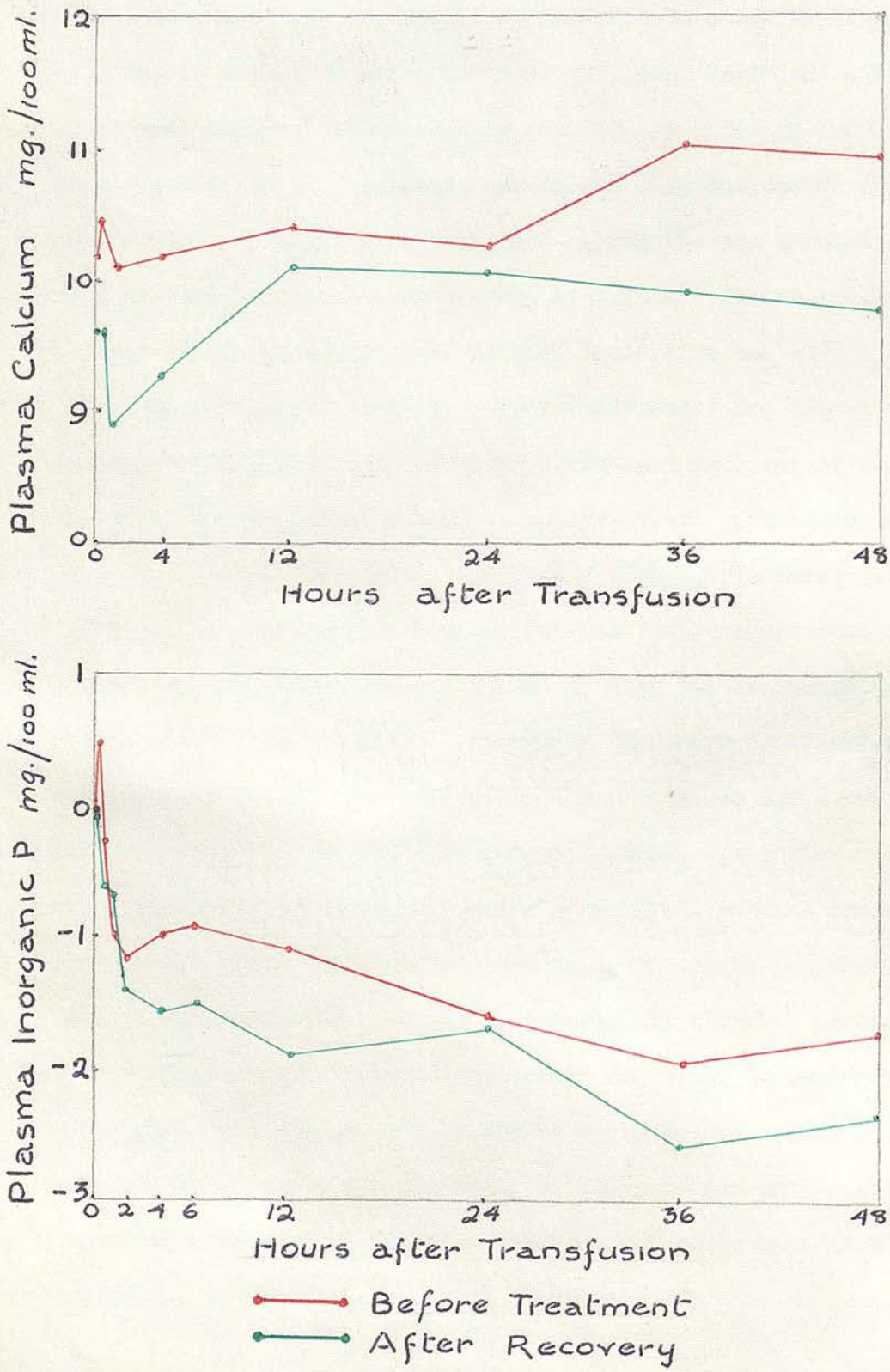
(b) Transfusions with plasma from calving cows

In Queensland the concentration of calcium in the plasma of lambs dropped during the first hour after the injection of bovine plasma (Figure 51), but no other significant changes or differences were noted in either the Queensland or Scottish experiments. In contrast, plasma inorganic phosphate concentrations dropped during the first hour after the transfusions in all experiments and remained depressed for six hours (Figure 52). The concentrations rose in the control groups at the twelve hour sample and thereafter remained fairly stable, but in lambs injected with plasma from parturient cows the levels remained depressed for twenty-four hours. Transfusions of plasma collected one to two days after calving produced variable responses.

Strict comparison of the control and other treatments is not easy in this figure because of the marked, though insignificant, differences between the treatment means of the groups. This effect is removed in Figure 53, where the changes in inorganic phosphate concentrations from pre-treatment values are shown. Transfusion experiments in Queensland with parturient andron-parturient plasmas produced almost identical curves and the difference between these at twenty-four hours after treatment was not significant. Highly significant differences were recorded in the Scottish experiments, where the transfusion of parturient plasma produced low phosphate values in the lambs at twelve and twenty-four hours after the injection. The use of post-partum plasma for transfusion was also associated with some depression of phosphate values over this period.

There were no significant changes in the plasma calcium concentrations

FIGURE 54 Plasma calcium concentrations and changes in the plasma inorganic phosphate concentrations in lambs following the intravenous injection of plasma collected from three cows with milk fever.



From Tables A66-A68.

TABLE XXIV

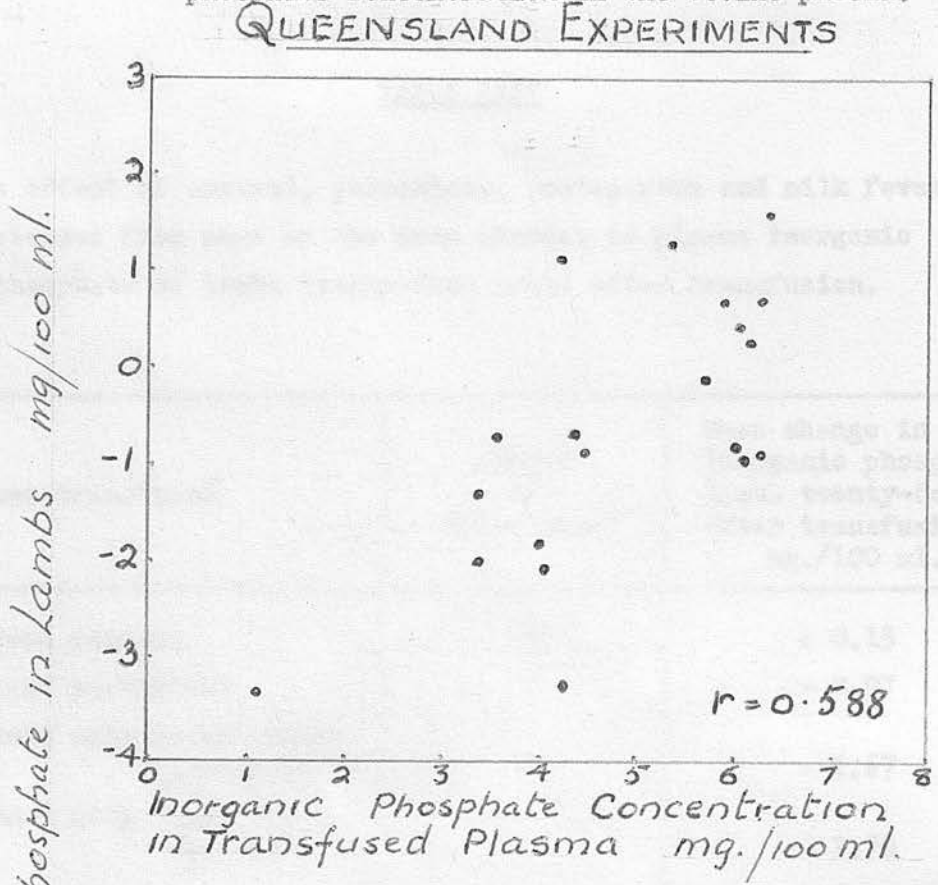
The effect of control, parturient, post-partum and milk fever plasmas from cows on the mean changes in plasma inorganic phosphate of lambs twenty-four hours after transfusion.

Plasma transfused	Number of Experiments	Mean change in plasma inorganic phosphate of lambs twenty-four hours after transfusion mg./100 ml.
Queensland control	8	+ 0.15
Queensland parturient	7	- 0.97
Queensland milk fever before treatment	3	- 1.67
Queensland milk fever after recovery	3	- 1.70
Scottish control	14	- 0.22
Scottish parturient	7	- 1.19
Scottish post-partum	6	- 0.73
All samples	48	- 0.66

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Between groups	6	16.9369	2.8228	2.38 *
Within groups	41	48.5911	1.1851	
Total	47	65.5280		

FIGURE 55 Correlation between the change in the plasma inorganic phosphate concentration of lambs twenty-four hours after the injection of bovine plasma and the inorganic phosphate concentration in the bovine plasma.



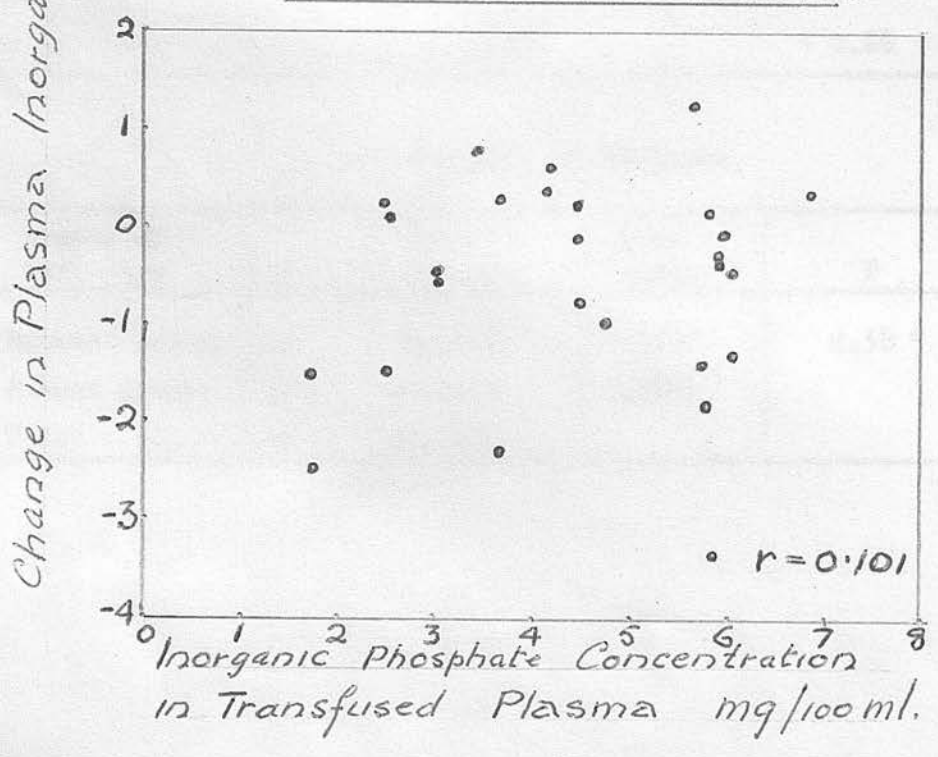


FIGURE 56 Correlation between the change in the plasma inorganic phosphate concentration of lambs twenty-four hours after the injection of bovine plasma and the inorganic phosphate concentration in the bovine plasma.

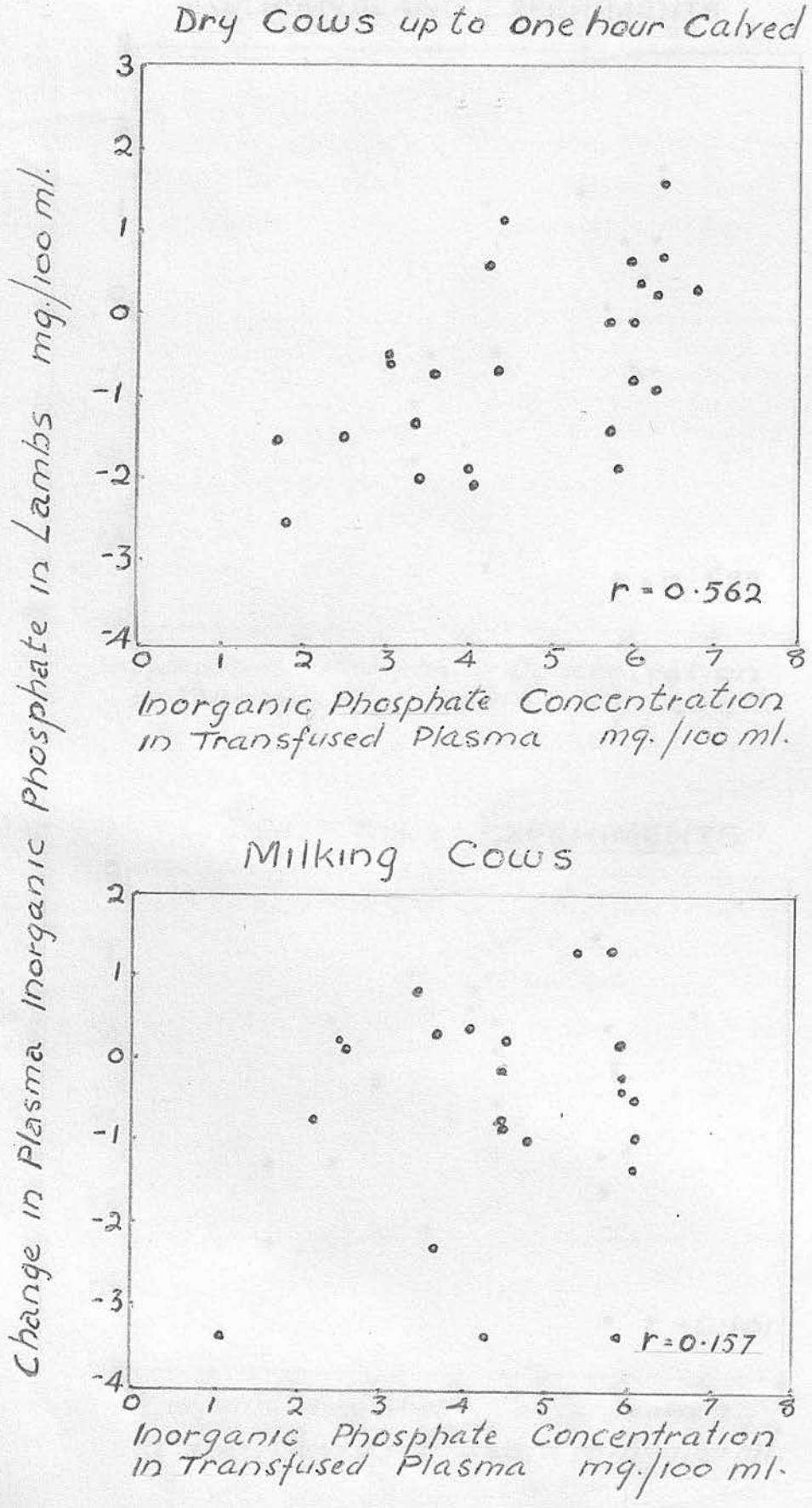


TABLE XXV

Correlation coefficients and regressions for the change in the concentration of inorganic phosphate in the plasma of recipient lambs twenty-four hours after the transfusion (y) and the inorganic phosphate concentration of the transfused plamsa (x).

Source of Data	n	Inorganic P. concentration in transfused plasma		Twenty-four hour change in plasma inorganic P. of lambs		Correlation coefficient	Regression equation	Standard deviation from regression
		Mean	s.d.	Mean	s.d.	r		Sy.x.
Queensland	22	4.44	1.606	-0.68	1.339	0.588 **	y = 0.49x-2.86	1.11
Scotland	27	4.44	1.504	-0.59	1.096	0.101		
Dry cows up to one hour calved	25	4.53	1.564	-0.58	1.103	0.562 **	y = 0.40x-2.44	0.93
Milking cows	24	4.36	1.528	-0.65	1.314	0.157		

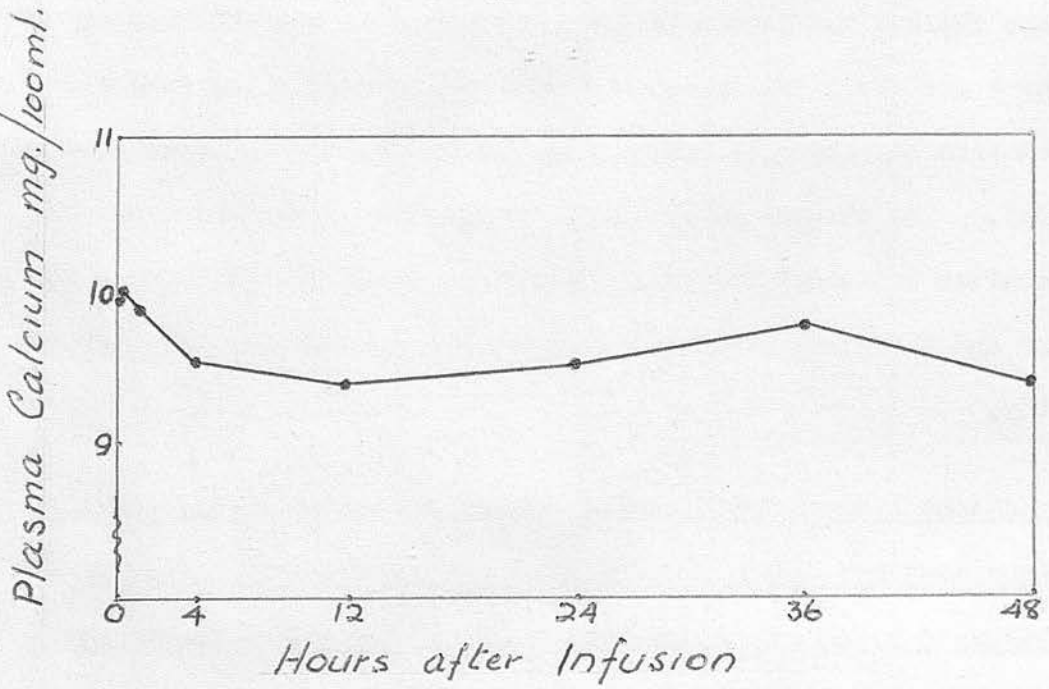
of the lambs injected with plasma taken from the Queensland milk fever cows (Figure 54), but the plasma phosphate values fell more heavily at twenty-four hours after the injections than when transfusions were made with plasma from any group of normal cows before, during or after calving (Table XXIV). The changes produced by transfusing plasma from the cows before and after treatment for milk fever were almost identical, even though the calcium and inorganic phosphate contents of the transfusates differed considerably.

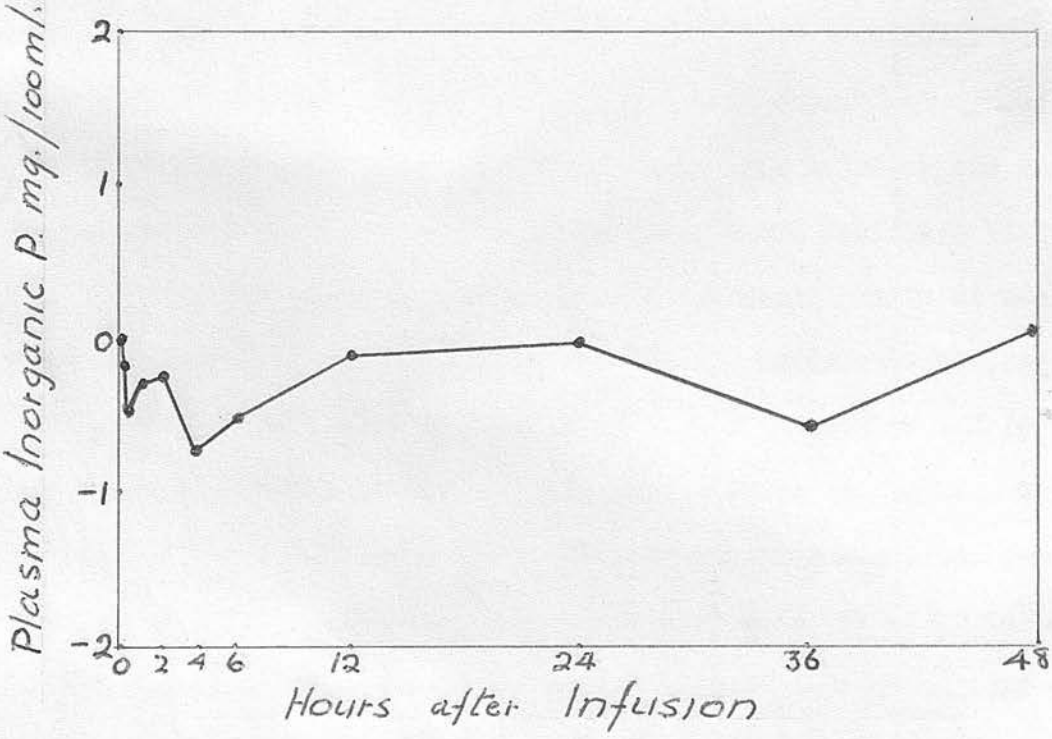
(c) Correlations between the inorganic phosphate concentrations in the transfused cow plasma and the changes in the concentration of inorganic phosphate in the plasma of the lambs twenty-four hours after the transfusion.

A tendency was noted in the Queensland experiments for low phosphate values to be associated with large depressions in the plasma phosphate levels of lambs twenty-four hours after transfusion but no similar tendency was observed in the Scottish experiments. Figure 55 illustrates this difference and Table XXV gives the statistical data. The difference between the correlations is almost significant at the five per cent level.

However, in Queensland all but one of the samples from the cows were collected either before calving or less than one hour after calving, but in Scotland most of the samples were collected at or after calving, so the data was regrouped into dry cows up to one hour calved and milking cows. Figure 56 and Table XXV show that the correlations for these two groups were very similar to those for Queensland and Scottish cows respectively.

FIGURE 57 Plasma calcium concentrations and changes in the plasma inorganic phosphate concentrations of lambs following the intravenous injection of inorganic phosphate solutions.





From Tables A69 and A70.

The correlation coefficient for dry cows was fractionally lower than for the Queensland cows due to the range of the responses in the lambs being slightly less. The regression equations are given in Table XXV together with the standard deviations from regression. These deviations indicate a slightly tighter grouping of the points about the regression line for the dry cows than for the Queensland cows.

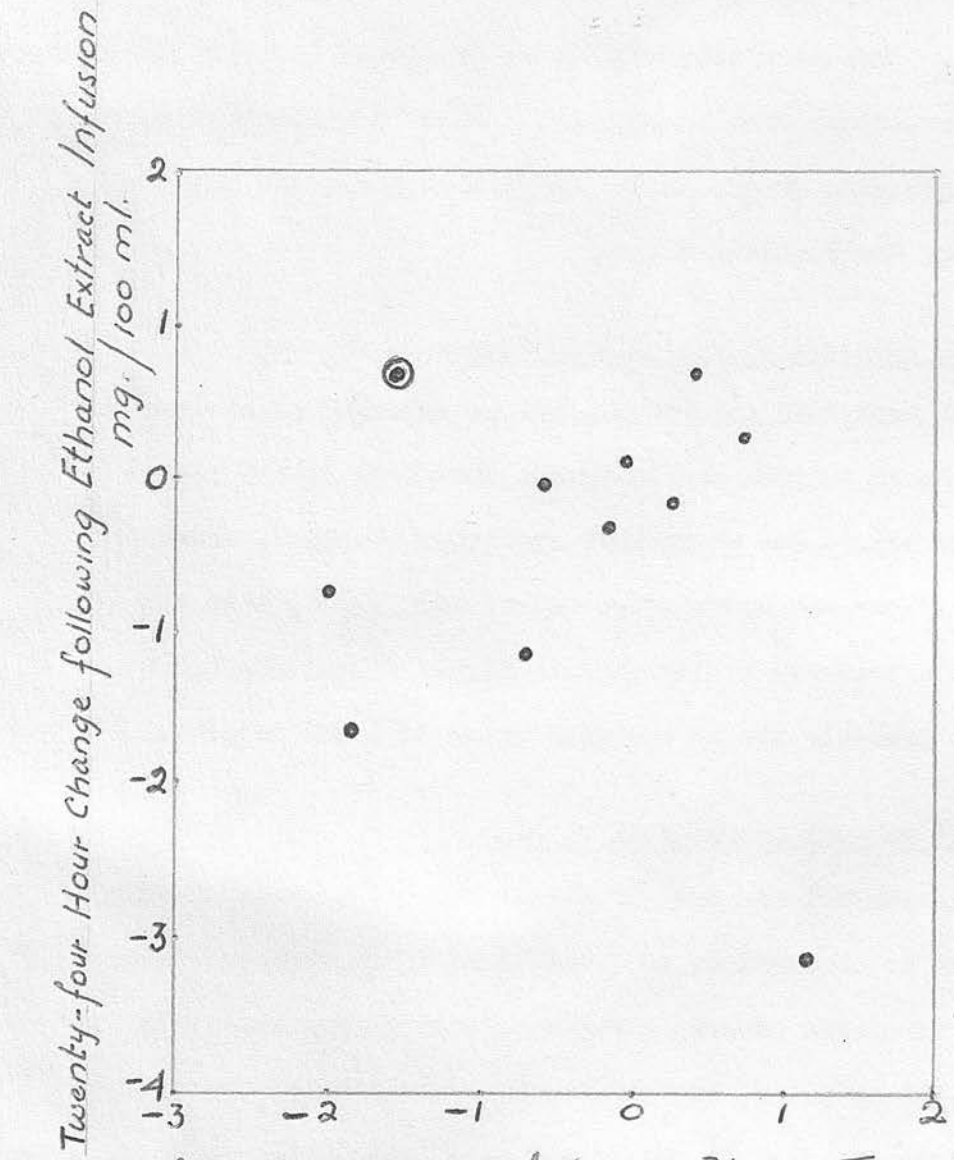
(d) The infusion of standard phosphate solutions.

The infusion of phosphate solutions produced no significant changes in the concentrations of calcium and inorganic phosphate in the plasma of the lambs (Figure 57). The changes in inorganic phosphate which were observed during the first six hours after transfusing plasma were not evident, apart from a tendency to lowering of values during the first half hour which was probably due to the withdrawal of blood samples.

(e) The infusion of ethanol extracts of plasma

The infusion of ethanol extracts of plasma was not associated with any change in plasma calcium values of those lambs which were examined. Nor did the plasma inorganic phosphate values change during the first few hours after the transfusion, but changes were observed at twenty-four hours similar to those produced by the transfusion of the corresponding plasmas (Tables A71-A73). Figure 58 shows the relation between the twenty-four hour response of lambs to plasma transfusion and to the infusion of approximately equivalent quantities of ethanol extracted plasma. The agreement is good when the technical difficulties and the natural variability of inorganic phosphate concentrations in plasma are

FIGURE 58 Correlation between the changes in the concentration of inorganic phosphate in the plasma of lambs twenty-four hours after the injection of bovine plasma and ethanol extracted bovine plasma.



◎ Mean of Two Plasma and Two Ethanol Extract Infusions

From Tables A64, A65 and A73.

considered. One aberrant result was found which cannot be explained, and the exclusion of this point gives a correlation coefficient of 0.532.

Discussion

In biological titrations it is usual to use groups of animals for each test to minimise the effect of natural variations in reponse. This was not possible in this series of experiments, except for a few cases where duplicate titrations were made, since the volumes of plasma required for transfusion were quite large and varied between 60 and 240 millilitres. As the haematocrit of donor cows at calving tends to be high, between 250 and 500 millilitres of blood had to be collected for each transfusion. The choice of dose was purely arbitrary and represents an injection equivalent to about ten per cent of the plasma volume of the lambs.

Replication would have been possible had smaller recipient animals been used, but a few infusions to other test animals such as rabbits were associated with high mortality rates. Moreover, unidentified hypothetical substances were being sought whose only suspected property was their influence on calcium and phosphate metabolism in ruminants and the smallest ruminants which were readily available were young lambs about three months old. Lambs of this age were old enough to allow serial sampling of their blood to be undertaken with only insignificant changes in plasma calcium and inorganic phosphate concentrations as the experiments with the administration of standard phosphate solutions show.

In the Queensland experiments where very young lambs were used there was a drop in the plasma calcium concentrations about one hour following the transfusions, but no other significant differences were observed. This may indicate that no hormone or other substance affecting calcium metabolism was present in the plasma of cows before, during or after calving, or in milk fever cases, but equally it could be the result of limitations in the experimental design. For example, the transfusion of a volume of plasma equal to ten per cent of the recipient's plasma volume might be expected to induce a response equal to one tenth of the change observed in the donor, which would hardly be detectable. Again, even if a substance interfering with calcium mobilisation or excretion were transfused, the homeostatic mechanisms of the lambs would possibly ensure that no change would occur in their plasma levels. As we have seen, it is difficult to produce a hypocalcaemia experimentally in cattle, other than in old lactating cows, where efficiency of mobilisation is probably impaired and where a steady demand for plasma calcium exists.

Plasma inorganic phosphate, however, appears to be much more labile. The plasma phosphate concentrations of lambs fell by about one milligram per cent during the first six hours following the transfusions, but this was not observed following either standard phosphate or ethanol extract infusions and so was possibly associated with the injection of foreign proteins. Later, at twelve and twenty-four hours after the plasma injections, some discrepancies appeared between the results of the Queensland and Scottish experiments, the most important of which was the

difference in the responses of the lambs to the transfusion of control and parturient plasmas.

The experiments in the two countries differed in a number of ways. The recipient lambs used in Queensland tended to be younger than in Scotland and some at least may have come from phosphate deficient pastures. In Scotland a wide variety of lambs of different breeds were used, the choice being governed by the smallest lambs available at any given season, but these differences do not appear to have affected the outcome of the trials. On the other hand, variations in management might account for the difference between the plasma phosphate changes of Queensland and Scottish cows around the time of calving, and this in turn might account for the failure to obtain significant differences between the lambs injected with control and parturient cow plasma in Queensland comparable to the changes found in Scotland. In the Scottish experiments all the cows had low levels of inorganic phosphate in their plasma at calving, but some of the Queensland cows had higher values at calving than before calving.

The positive correlation in the Queensland experiments and the lack of correlation in the Scottish experiments between the phosphate concentration in the donor plasma and the twenty-four hour lamb response might indicate different physiological mechanisms in the cows of the two countries, but this would be very difficult to justify and there is an alternative explanation based on the times of collection of blood from the cows. Figure 56 demonstrates a significant correlation for the dry cows, but not for the milking cows, between the inorganic phosphate content of the

transfused plasma and the response of the lambs twenty-four hours after the transfusion. The figure for dry cows is similar to that of the Queensland cows (Figure 55), the correlations being almost identical, but it is not overwhelmingly influenced by the Queensland data, since ten of the twenty-five points are from Scottish experiments. The standard deviation from the regression line for the dry cow data is a little less than that for the Queensland data (Table XXV).

Thus a tentative conclusion might be reached that the transfusion of cow plasma into lambs which is collected before or up to one hour after calving will produce changes in the inorganic phosphate content in the plasma of the lambs which are positively correlated with the phosphate content of the transfusate. Plasma obtained from the milking cows may produce variable responses, when injected into lambs, which are independent of the phosphate content of the transfused plasma. This suggestion fits in with the results of Section III, where no correlation was found between feed intake and plasma phosphate levels before calving, but where plasma inorganic phosphate concentrations were positively correlated with both feed intake and milk yield after calving in the older dows. Most of the cows used in these experiments were at their third or subsequent calving.

In the dry cow experiments it is obvious that the actual concentration of phosphate in the transfusate can have little if any effect on the phosphate concentration in the plasma twenty-four hours after transfusion. The transfusate totalled about ten per cent of the lambs' plasma volume, so a difference of one milligram per cent between the phosphate contents

of the transfused plasma and the lamb's plasma would have produced a change of only 0.1 mg. per cent in the plasma of the lambs by simple dilution and the effect would have been seen immediately. As the regression equation in Table XXV shows, a change of 1.0 mg. per cent in the inorganic phosphate of the transfused plasma was associated with a change of 0.4 to 0.5 mg. per cent in the lambs plasmas. Thus some other factor being carried in the plasma may regulate actively, not passively, the inorganic phosphate concentrations in the plasmas of parturient cows. The activity of this substance in the blood of cows seemed to occur about the time that calcium and phosphate metabolism were under stress and to persist for some time after the return of blood inorganic phosphate values to normal, as for example, in cows one day calved or recovered from milk fever. This substance possesses a number of properties in common with parathyroid hormone. In addition to its effect on blood inorganic phosphate, there was the twelve to twenty-four hour delay in response to the injection and the extraction of the active substance from plasma with ethanol, which indicates it is neither albumin nor globulin.

After calving the plasma phosphate changes are quite variable and young cows especially show a marked increase in phosphate levels within a few hours of calving, but older cows may have depressed phosphate values for one or even two days (Moodie et al., 1955). Observations on the development of milk fever show that the plasma phosphate levels remain depressed between calving and the onset of the condition and a satisfactory response to calcium therapy or udder inflation is associated

with a definite improvement in plasma phosphate levels whereas a poor response is associated with failure in the treatment (Marr et al., 1956).

These observations suggest that a deeper study of the aetiology of the changes in plasma phosphates of cows about the time of calving might be rewarding and therefore the effects of the transfusion of plasma to lambs are of potential interest. However, because of the similarity of the responses by lambs to plasma collected from milk fever cows before treatment and after recovery, the lack of association between lamb response and phosphate content of the plasmas of cows after calving, the lack of information on the exact significance of plasma phosphate changes at calving and the failure to elicit any change in the plasma calcium levels of the lambs after transfusion, the experiments were temporarily abandoned at this stage. Nevertheless, it seems possible that the active principle can be readily extracted with ethanol and its subsequent purification and identification may not be too difficult should this prove desirable.

Summary

A series of blood samples was collected from cows in Queensland and Scotland before, during and after calving and the effect of injecting lambs with the plasma separated from these samples was studied. Twenty-two plasma transfusions were made in Queensland and twenty-eight in Scotland. In each experiment between two and three millilitres of plasma were injected intravenously per pound liveweight and blood samples were collected from the lambs before and at frequent intervals for forty-eight hours after the injection.

The transfusions had no effect on the concentrations of calcium in the plasma of the lambs, but the injection of plasma of low phosphate content collected from cows before or at calving seemed to induce a depression in the plasma phosphate concentrations in the lambs twelve to twenty-four hours later. The effect of transfusing plasma collected from milking cows was variable. Samples were collected from three cows in Queensland before treatment and after recovery from milk fever. All these samples depressed the plasma phosphate concentrations in the lambs very markedly, even though the phosphate contents of the recovery samples were high.

There was evidence that the plasma inorganic phosphate concentrations of cows at calving in Queensland behaved differently from those of cows in Scotland.

SECTION VIII

THE EFFECT ON PLASMA CALCIUM AND INORGANIC PHOSPHATE OF HORMONES ASSOCIATED WITH PARTURITION AND THE ONSET OF LACTATION

During the course of the experiments reported in Section III, one cow became excited while a blood sample was being withdrawn and the lactic acid content of this sample was subsequently found to be high. It was therefore decided to observe the effect of excitement on the blood composition of milking cows, since any release of adrenaline might account for the stasis of the alimentary canal at calving and the associated elevated blood glucose (Alexander and Moodie, 1960).

In Section VII an attempt was made to detect an active substance in the blood of calving cows which would depress the plasma calcium and phosphate levels of lambs, but no activity was found which would neatly fit the observations made on calving cows. The alternative approach is to examine the effect on the biochemical processes and alimentary tract activity of ruminants of those factors which may be present in the circulation at the time of calving.

Parturition and the onset of lactation are associated with changes in the secretion of a number of hormones. In the cow the circulating levels of progesterone fall about two weeks before term and the hormone balance shifts in favour of oestrogen (Meites, 1959; Short, 1960). It is into this environment that the specific hormones associated with parturition and the onset of lactation are secreted, for example,

oxytocin, relaxin and prolactin (Cole and Cupps, 1959). At the same time, any hormones originating from the foetus or the placenta are withdrawn from the maternal circulation.

Fitzpatrick (1960) has shown that in cattle the sensitivity of uterine muscle to oxytocin is greatly enhanced in the presence of oestrogen, and that non-parturient oestrogen sensitised animals and parturient animals respond equally to oxytocin injections. He has also shown that the concentration of oxytocin in the jugular blood of cows and sheep is increased twenty to one thousandfold during parturition (Fitzpatrick 1961a; Fitzpatrick and Walmsley, 1962) and that the uterus responds to vasopressin. Cows sensitised with oestrogen may show relaxation of the cervical muscles following varying doses of relaxin, (Graham and Dracy, 1953), but the level of naturally occurring relaxin in the blood of cows at calving has not apparently been assayed. In ewes there is no evidence of an increased concentration of relaxin in the blood during late pregnancy (Hall, Hoare and Turner, 1962). The changes in prolactin secretion during the initiation of milk secretion are the subject of dispute but it would seem that its activity is dependent on oestrogens (Folley, 1956; Meites, 1959).

The concentrations of oxytocin in the blood of normal calving and milk fever cows were estimated by Bell and Morris (1934a; 1934b), who reported relatively high values for milk fever cases. They attempted to induce milk fever in one cow by administering pituitrin, but without success. Oxytocin is not, however, without biochemical effect, since its administration to women soon after parturition has been associated

with a drop in blood glucose levels (Burt, Leake and Dannanburg, 1963), and with a hyperglycaemia in dogs and rabbits (Mirsky, 1962; Chaudhury and Nayyar, 1962; Burt, Leake and Dannenburg, 1964).

In view of these observations, it was decided to study the effect of posterior pituitary hormones and oestrogens on the blood composition of cattle and sheep. In some sheep experiments, the effect of these hormones on the absorption of calcium and phosphate was estimated by observing hepatic veno-arterial differences before, during and after hormone infusions.

Materials and Methods

Experiments were carried out on Ayrshire cows, and on Suffolk sheep which were one to two years old and weighed fifty to sixty kilograms. Four sheep were prepared for hepatic venous blood sampling as described in Section I and with a carotid loop (Schambye, 1951).

Blood sampling:- Arterial samples were obtained by inserting a 2.5 cm. x 20 B.W.G. serum needle into the carotid artery and allowing the blood to flow freely. Peripheral venous blood was obtained from the jugular vein by direct venipuncture and hepatic venous blood was withdrawn from the posterior vena cava of prepared sheep, as described in Section I.

Analyses:- The blood analyses were carried out as described in Sections III and VI, except that where whole blood was analysed for calcium and magnesium content the methods were modified as described in Section I.

Rumen movements:- These were recorded as described in Section I.

Hormones:- The hormones used in these experiments were Pituitary

TABLE XXVI

The effect of excitement on the blood constituents of six cows

	Before		After	
	Mean	S.D.	Mean	S.D.
Serum calcium (mg./100 ml.)	10.43	± 0.69	10.52	± 0.54
Whole blood inorganic phosphate (mg./100 ml.)	5.39	± 0.65	5.32	± 0.42
Whole blood citric acid (mg./100 ml.)	2.95	± 0.54	2.81	± 0.46
Whole blood glucose (mg./100 ml.)	47.2	± 5.68	46.8	± 7.17
Whole blood lactic acid (mg./100 ml.)	3.27	± 0.61	9.18	± 3.45 *
Whole blood pyruvic acid (mg./100 ml.)	0.77	± 0.06	1.40	± 0.48 †

Variance Ratios *F = 31.8 Sig. at 1 per cent.
†F = 64 Sig. at 1 per cent.

(Posterior Lobe) Extract, B.P.C., Purified Oxytocic Principle containing ten oxytocic units per millilitre (Armour Pharmaceutical Company Limited, Eastbourne, England) and Vasopressin Injection B.P. containing twenty units of pressor activity per millilitre (Pitressin, Parke Davis and Company, Hounslow, near London). The oestrogen was administered as stilboestrol. In the infusion experiments the hormone preparations were diluted with water or, in the case of stilboestrol, with a small quantity of olive oil. The infusions were made with a continuous injector apparatus (Palmer, London) and an indwelling nylon catheter.

Results

(a) The effect of excitement and exercise

Six cows were stimulated for a period of five to ten minutes by varying combinations of chasing around a yard, unusual noises and pricking of the hindquarters. The results are shown in Table XXVI. No changes were observed in serum calcium or blood inorganic phosphate, citric acid or glucose. The mean concentration of blood lactic acid, however, increased to three times its original value while that of pyruvic acid doubled and the magnitude of these changes was apparently dependent on the degree of muscular activity. For example, four cows which were exercised in addition to other stimulation showed three to fourfold increases in lactic acid. Of the two stimulated by pricking only, one showed marked muscular tremors and the lactic acid rose from 3.3 to 6.1 mg. per cent, while the other showed no tremors and no increase in lactic or pyruvic acids.

TABLE XXVII

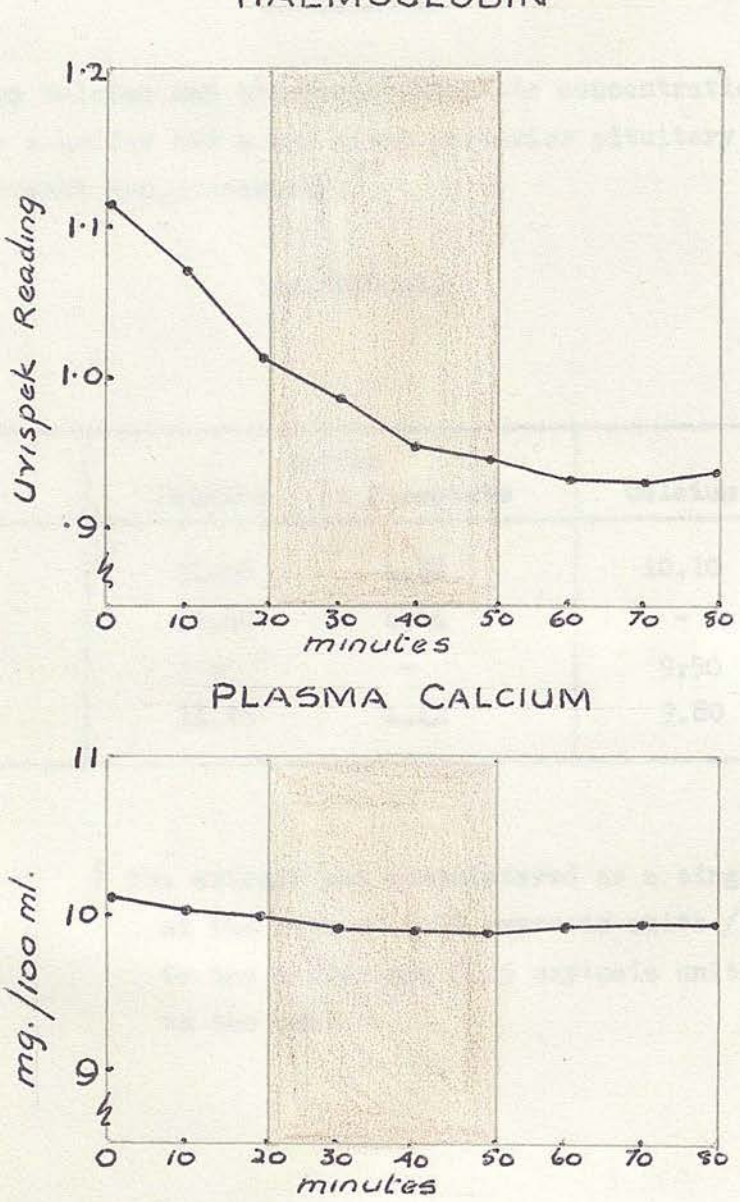
Plasma calcium and inorganic phosphate concentrations in a heifer and a cow given posterior pituitary extract subcutaneously.†

mg./100 ml.

Hours after injection	Heifer		Cow	
	Calcium	I.Phosphate	Calcium	I.Phosphate
0	11.65	4.32	10.10	5.67
½	10.60	4.44	-	-
1	-	-	9.50	4.79
2	11.15	4.42	9.80	5.04

† The extract was administered as a single dose at the rate of 0.15 oxytocic units / kg. to the heifer and 0.25 oxytocic units / kg. to the cow.

FIGURE 59 The effect of administering purified oxytocic principle (450 mu./min) intravenously on the jugular blood haemoglobin and plasma calcium concentrations of sheep.



From Table A74.

(b) The effect of posterior pituitary extract on cattle

Two animals were injected subcutaneously with Pituitary (Posterior Lobe) Extract B.P.C. containing 10 oxytocic units per millilitre. An unserved heifer was given 0.15 oxytocic units per kilogram and a non-pregnant cow received 0.25 oxytocic units per kilogram. No change in rumen movements was detected in either animal during the first two hours after the injection (Figures A6 and A7). Plasma calcium and inorganic phosphate concentrations are shown in Table XXVII.

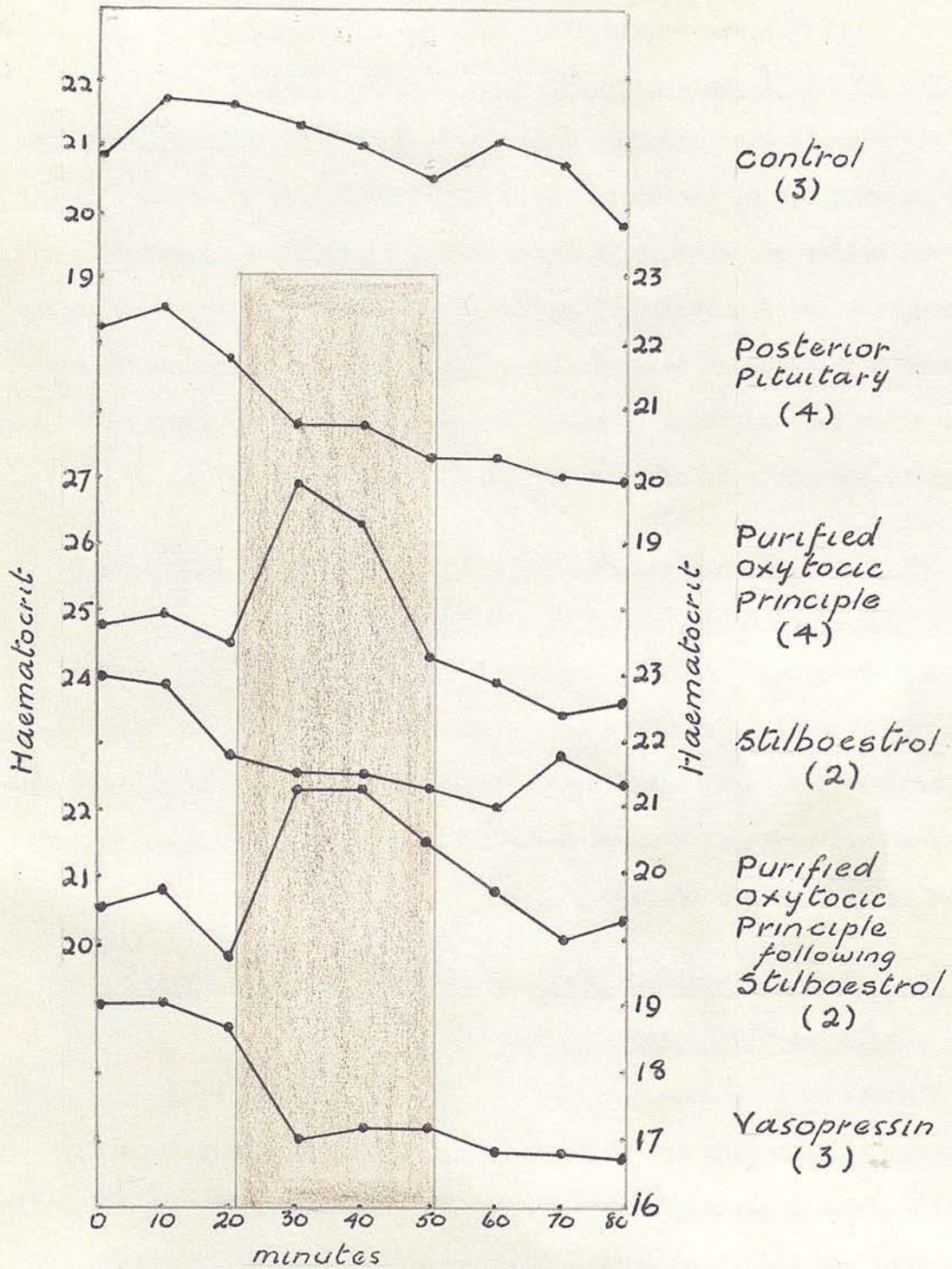
(c) The effect of purified oxytocic principle on the composition of the jugular blood of sheep

Six sheep were injected intravenously with 450 mu. of purified oxytocic principle per minute for thirty minutes and jugular blood samples were collected before, during and after the infusion. The hormone appeared to affect neither the downward trend of the haemoglobin values nor the plasma calcium values (Figure 59).

(d) The effect of posterior pituitary hormones and oestrogen on the arterial blood composition of sheep

Figures 60 to 64 show the haematocrit and haemoglobin readings and the calcium, magnesium and inorganic phosphate concentrations in the carotid arterial blood of sheep during control experiments (no injection), and during the injection of posterior pituitary extract, purified oxytocic principle (non-sensitised and oestrogen sensitised sheep), stilboestrol and vasopressin. The posterior pituitary extract and purified oxytocic principle were injected at the rate of 450 milliunits oxytocic principle per minute, stilboestrol at 20 micrograms in 0.15

FIGURE 60 The effect of administering posterior pituitary hormones and oestrogen on the arterial haematocrit of sheep.

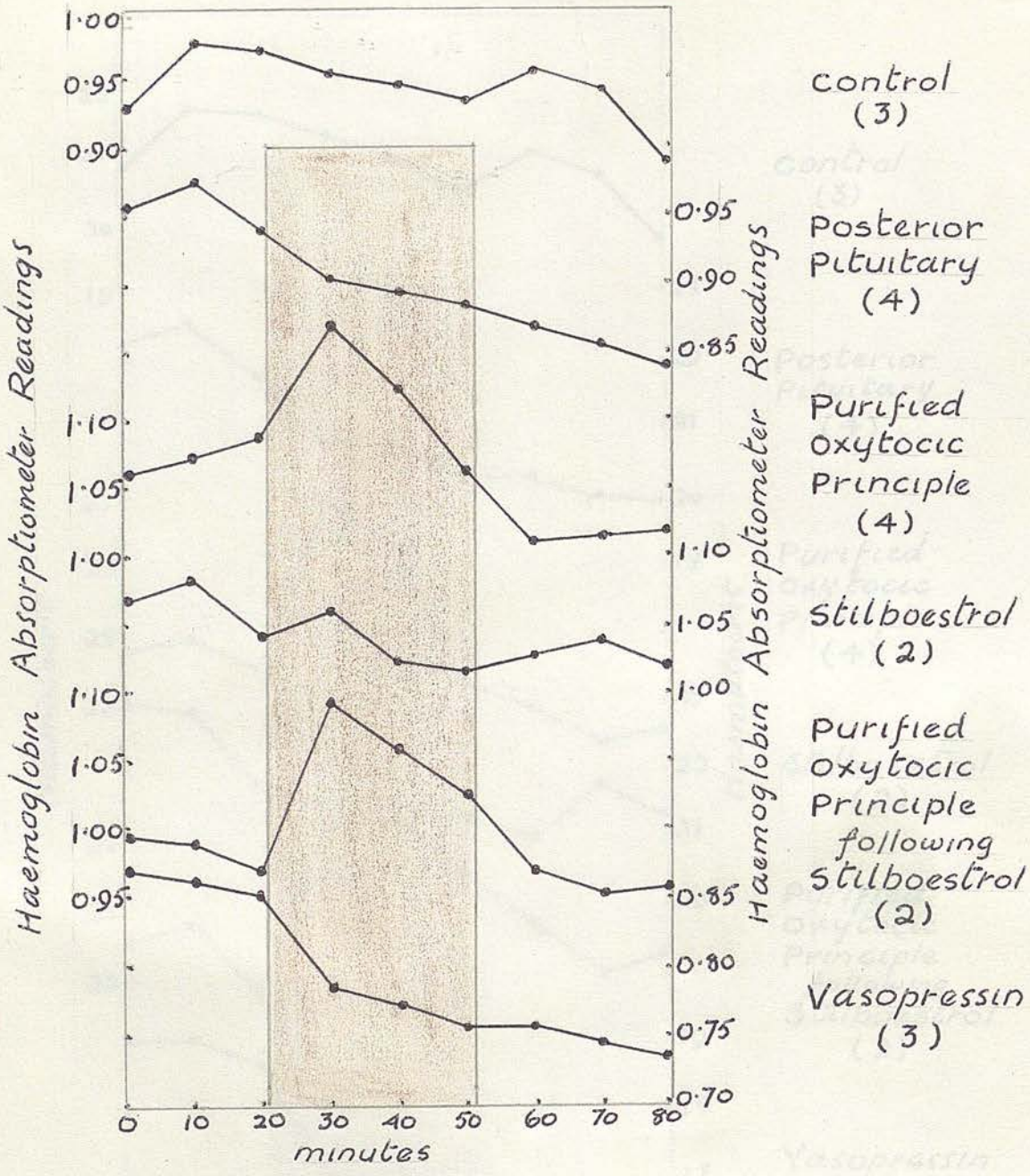


Shaded area — period of hormone injection
For dose rates see text.

Number of experiments shewn in brackets.

From Table A75.

FIGURE 61 The effect of administering posterior pituitary hormones and oestrogen on the arterial haemoglobin readings of sheep.

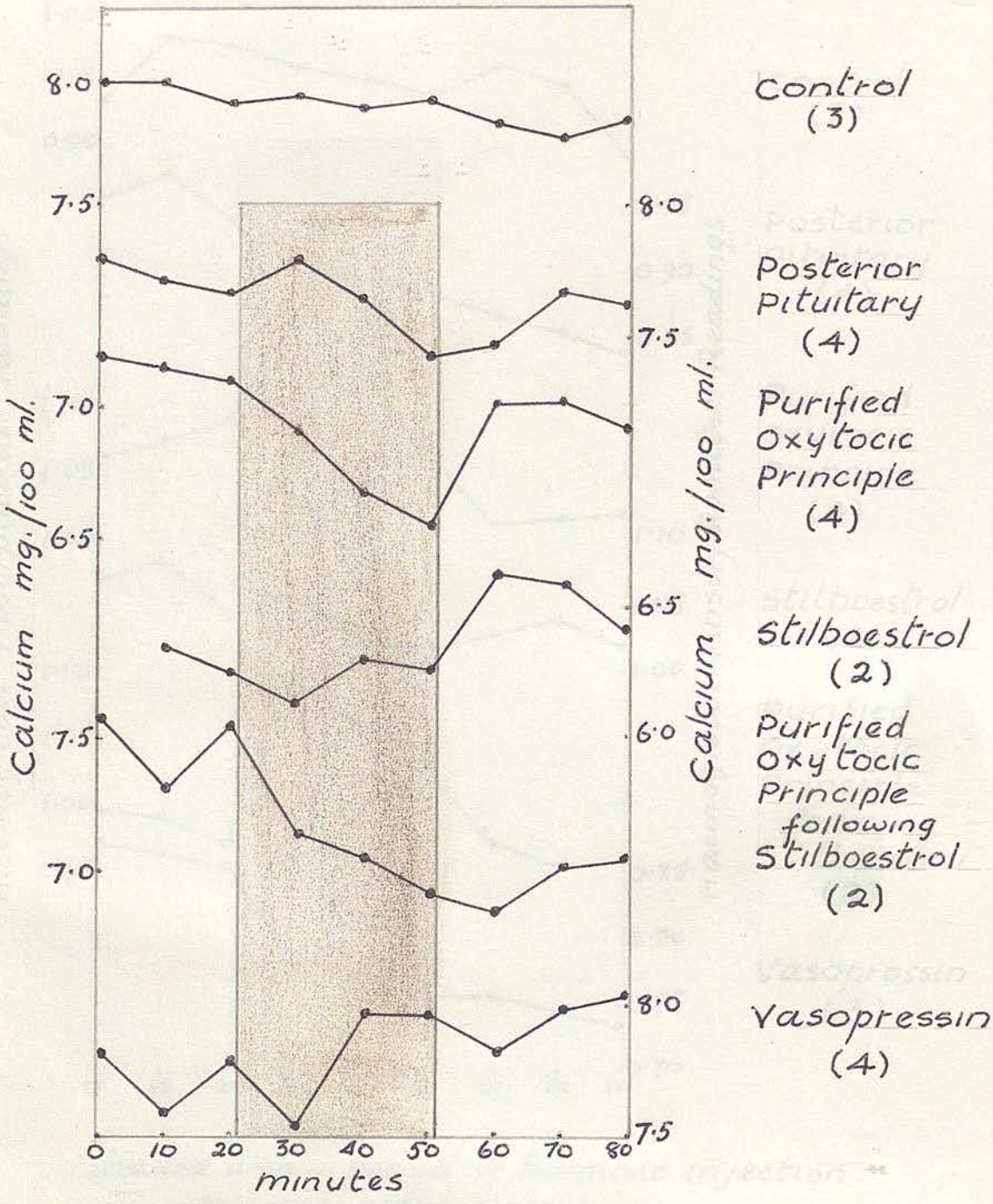


Shaded area – period of hormone injection
For dose rates see text.

Number of experiments shewn in brackets.

From Table A76.

FIGURE 62 The effect of administering posterior pituitary hormones and oestrogen on the arterial blood calcium concentrations of sheep.



Shaded area – period of hormone injection

For dose rates see text

Number of experiments shewn in brackets

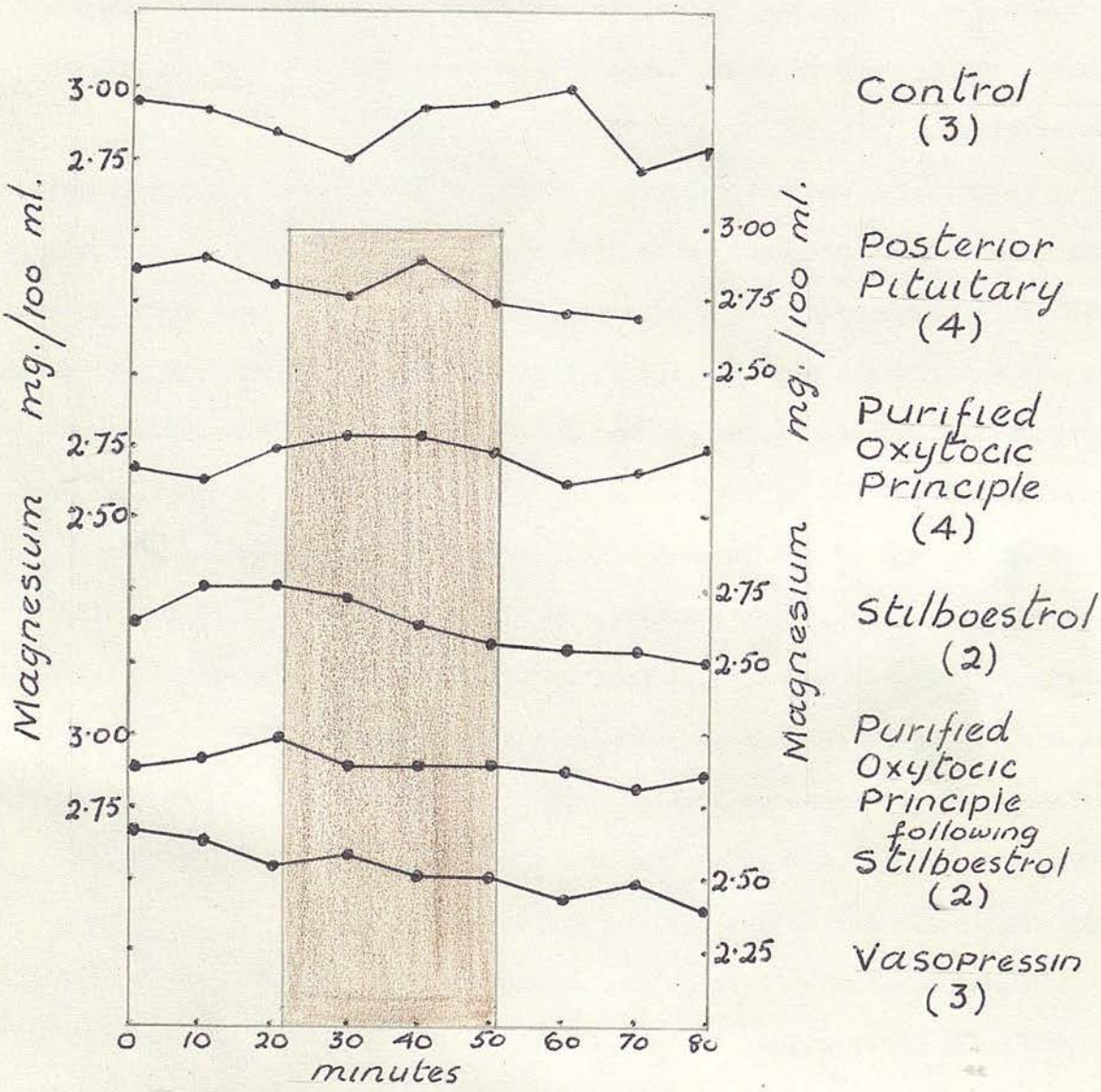
From Table A77.

millilitres of olive oil per minute and vasopressin at 450 milliunits of pressor activity per minute. Each experiment consisted of three periods - a preinjection phase, an injection phase and a post-injection phase, during each of which three samples were collected at ten minute intervals.

Haematocrit and haemoglobin readings:- In the control experiments the haematocrit and haemoglobin readings tended to drop by about ten per cent over the period of the experiments and similar changes were observed in the experiments with posterior pituitary extract. The administration of purified oxytocic principle caused a sharp rise in haematocrit and haemoglobin but the effect seemed to fade as the infusion continued so that by the end of the injection the haematocrit had returned to its preinjection level. In the two sheep sensitised with oestrogen the changes were perhaps a little more marked than in experiments on the same sheep prior to sensitisation (see Tables A75 and A76). Administration of vasopressin caused an initial depression of haematocrit and haemoglobin readings, but the effect of this was also transitory. Stilboestrol apparently did not affect the red cell volume.

Blood calcium:- Statistical examination of the data gave a highly significant interaction (Table A77) which indicates that one or more of the treatments were producing a significant effect. In the control experiments all three sheep had constant levels of blood calcium and values dropped by only three per cent over the course of the observations. The calcium concentrations in the posterior pituitary experiments were much more variable than in the control experiments and this probably

FIGURE 63 The effect of administering posterior pituitary hormones and oestrogen on the arterial blood magnesium concentrations of sheep.

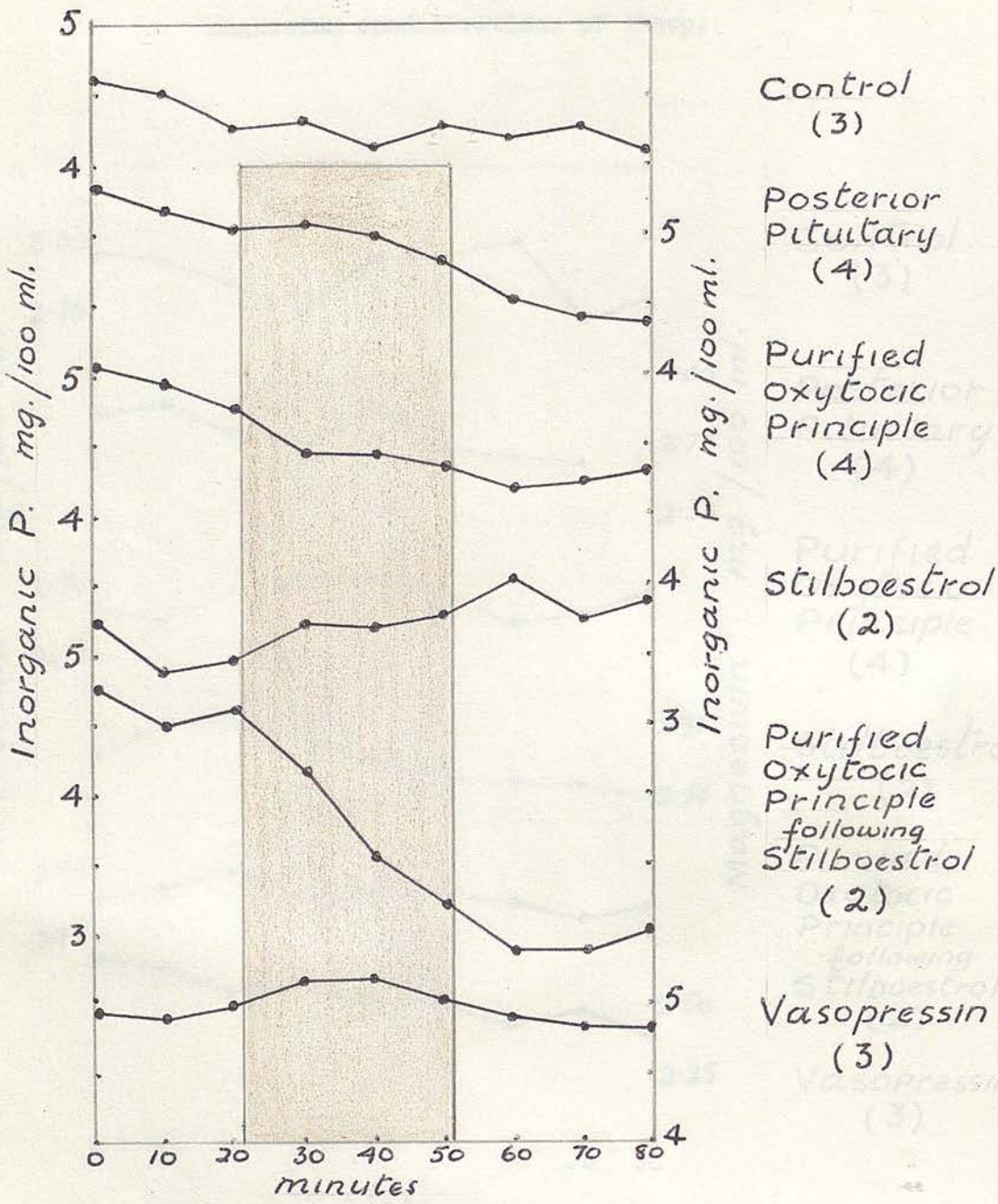


Shaded area — period of hormone injection
For dose rates see text

Number of experiments shown in brackets

From Table A78.

FIGURE 64 The effect of administering posterior pituitary hormones and oestrogen on the arterial blood inorganic phosphate concentrations of sheep.



Shaded area — period of hormone injection
For dose rates see text

Number of experiments shewn in brackets

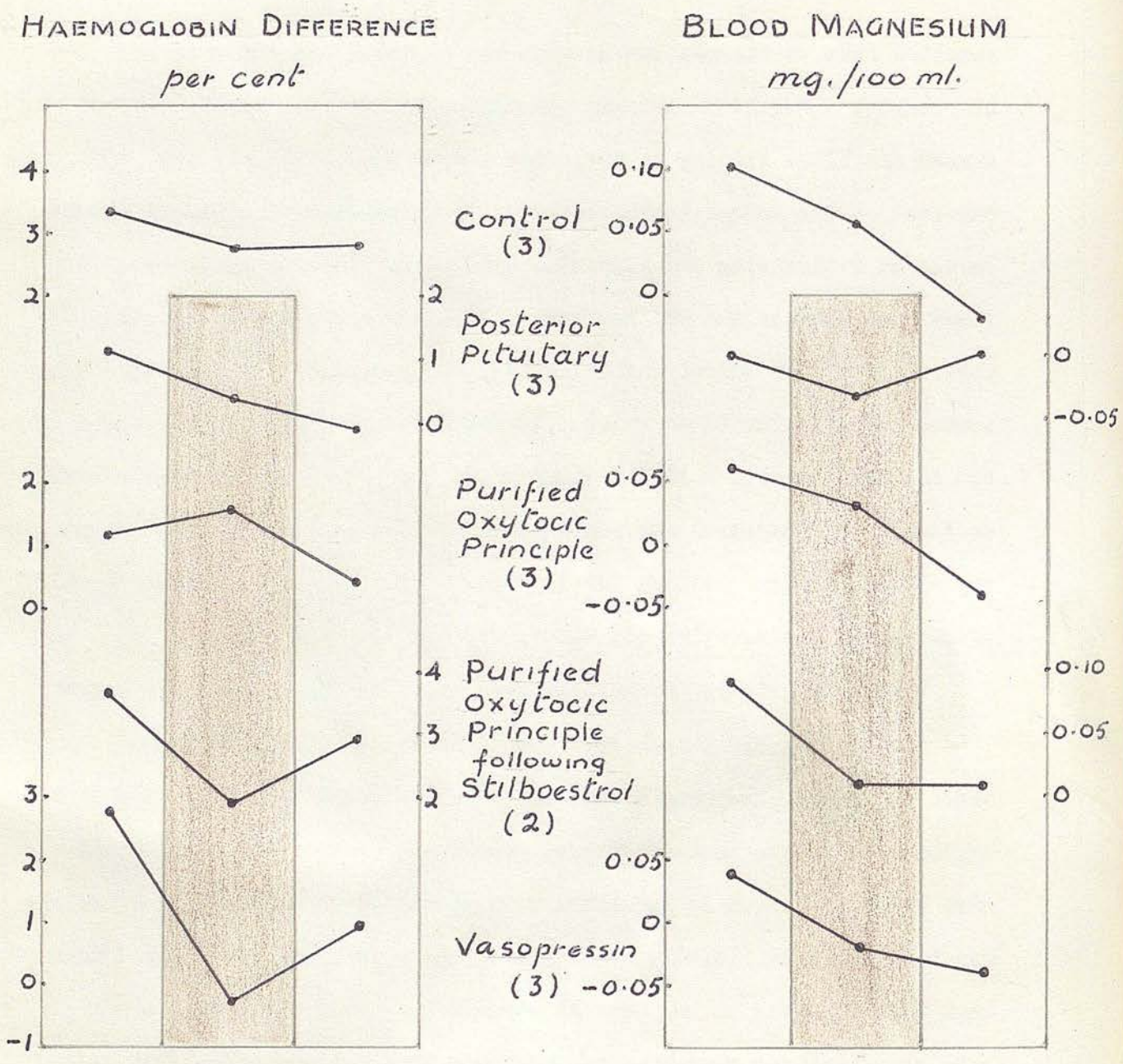
From Table A79.

resulted from an interaction between the oxytocin and vasopressin components. Purified oxytocic principle injected for thirty minutes caused the blood calcium to drop from a mean of 7.1 mg. per cent to 6.55 mg. per cent at the end of the injection. The increase in arterial calcium levels on withdrawing the injection was rapid. In the oestrogen sensitised animals the changes were a little greater and seemed to persist after the end of the injection. Vasopressin, on the other hand, tended to raise the blood calcium levels towards the end of the injection, but the results were a little erratic and require to be interpreted with caution. Stilboestrol was administered to two animals but did not produce any change in blood calcium levels which cannot be accounted for by the effect of injecting olive oil alone.

Blood magnesium:- No changes were noted in blood magnesium levels.

Blood inorganic phosphate:- The differences among the changes in blood inorganic phosphate in the control, posterior pituitary, purified oxytocic principle (non-sensitised sheep) and vasopressin experiments were small. Posterior pituitary extract and purified oxytocic principle may have produced slightly greater reductions in blood phosphate concentration than were observed in the control experiments, while vasopressin may have caused slight increases in inorganic phosphate values. The major change was noted in the two sheep sensitised with stilboestrol, where blood inorganic phosphate concentrations fell by more than 1.5 mg. per cent during the course of oxytocin administration and were only beginning to recover thirty minutes after the end of the injection. Stilboestrol produce no change in blood inorganic phosphate concentrations.

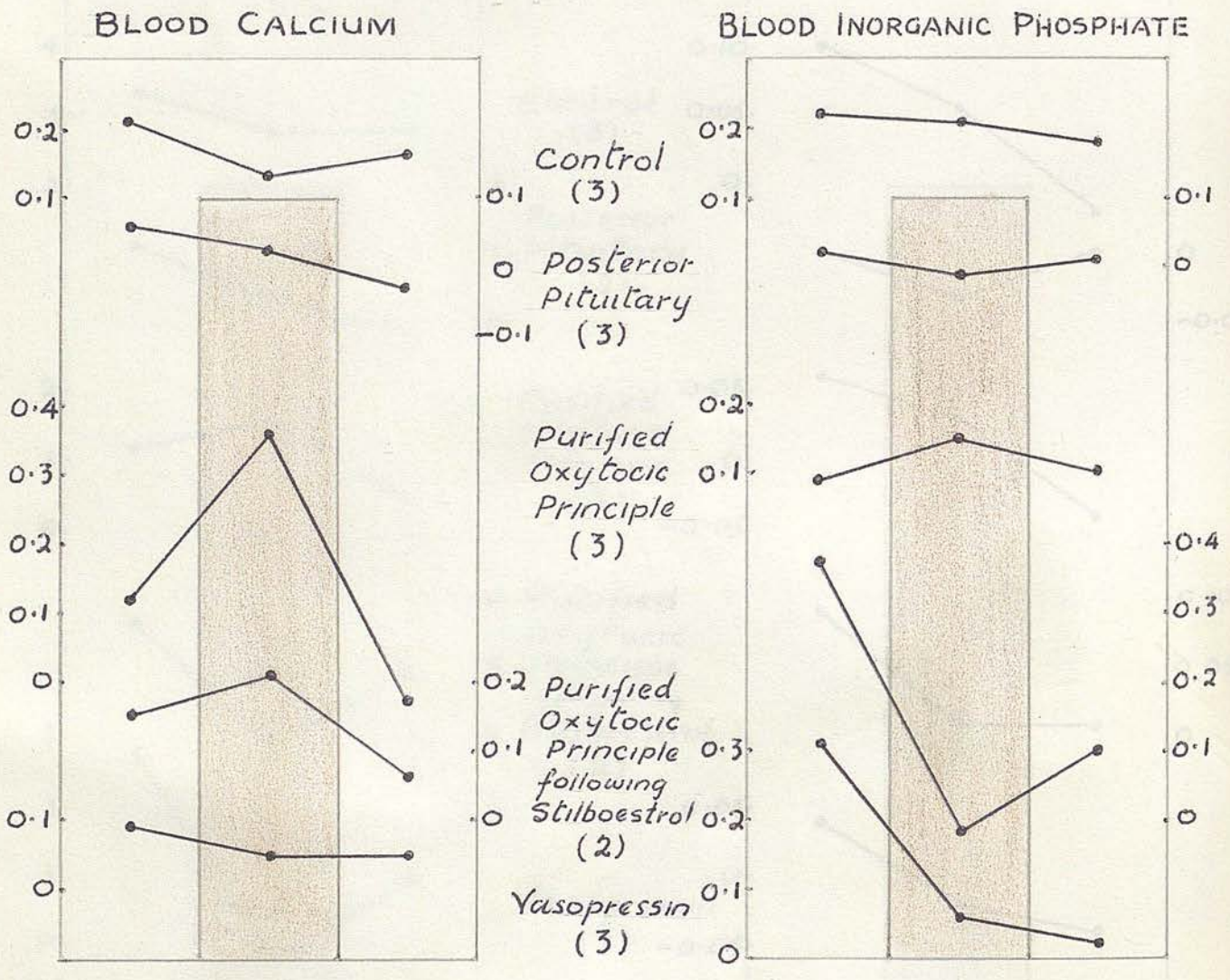
FIGURE 65 The effect of administering posterior pituitary hormones on the hepatic veno-arterial differences of sheep.



Shaded area — period of hormone injection
For dose rates see text
Number of experiments shewn in brackets

From Tables A80 and A82.

FIGURE 66 The effect of administering posterior pituitary hormones on the hepatic veno-arterial differences of sheep.



Shaded area — period of hormone injection
For dose rates see text
Number of experiments shewn in brackets

From Tables A81 and A83.

(e) Changes in the composition of blood passing from the arterial system to the hepatic veins of sheep during the infusion of posterior pituitary hormones

The V-A differences for haemoglobin (percentage haemoglobin differences) and corrected values for calcium, magnesium and inorganic phosphate are shown in Figures 65 and 66. In each treatment the data are grouped into single mean values for the three periods of the experiments (pre-injection, injection and post-injection) since the data are rather limited.

Percentage haemoglobin differences:- The percentage haemoglobin differences were always lower in the post-injection period than in the pre-injection period. During the injection there was little difference between the control and posterior pituitary extract experiments. Purified oxytocic principle in the non-sensitised sheep may have produced a slight increase in water absorption, but in oestrogen sensitised sheep the percentage haemoglobin difference may have been depressed (there is insufficient data to reach a firm conclusion). The effect of vasopressin was quite clear and virtually no absorption of water was detected during the period of infusion.

Magnesium corrected V-A differences:- No effect on magnesium absorption was observed apart from a general downward trend in values in all experiments.

Calcium corrected V-A differences:- No significant changes were observed in the V-A differences although higher values were recorded in two of the three non-oestrogen sensitised animals injected with

purified oxytocic principle.

Inorganic phosphate corrected V-A differences:- No changes were observed in the control, posterior pituitary and purified oxytocic principle experiments with non-sensitised sheep. In the sensitised sheep, however, there was evidence of a very marked drop in inorganic phosphate absorption to a mean value of zero. A similar change, although not quite so marked, was observed during the vasopressin administration.

Discussion

One of the major problems in experiments of this nature is to assess the amount of stimulus which should be applied to reproduce the natural event which is being studied. For this reason the experiments recorded in this section must be interpreted with some caution, for although they may offer an explanation for some of the biochemical changes which were observed in the calving cows in Section III, they do not provide an adequate proof.

The results in Table XXVI indicate that excitement accompanied by a certain amount of muscular activity may produce increased levels of lactic and pyruvic acids in the circulation comparable to those found in calving cows, so it would seem that changes in these blood constituents in cows at calving could be accounted for by excitement and uterine and abdominal muscular activity, and by the struggling associated with milk fever. In normal calving cows the increase in blood glucose concentrations seemed to precede the changes in lactic acid, which suggests that the glucose changes were not the result of excitement or muscular activity.

The appropriate dose of posterior pituitary hormone is difficult to estimate as it varies with the physiological state of the animal. The commonly stated dose of 100 oxytocic units (Brit. Vet. Codex 1953) is believed by Fitzpatrick (1960) to be much too high for the calving cow, where the uterus is sensitised to oxytocin and where in addition a considerable quantity of hormone is already in the circulation. In the non-pregnant animal relatively higher doses are necessary to produce an effect on the uterus and this governed the dose of posterior pituitary extract given to the two cows to study its effect on alimentary mobility. As Figures A6 and A7 show, there were no noticeable effects on rumen movements.

The desirability of using whole blood when estimating the absorption of minerals from the alimentary canal by V-A differences was discussed in Section VII and this is the reason for estimating the arterial concentrations of calcium, magnesium and inorganic phosphate on blood. The values obtained for calcium in particular will be influenced by the volume of cells in each aliquot taken for analysis and this may have accounted for some of the differences among the mean blood calcium concentrations of different animals. It does not explain the serial differences, for although the cell volume increased or decreased by two or three percentage units in some experiments, the effect of this on the volume of plasma taken for estimation in each aliquot of blood would only be of the same order. For example, if the haematocrit increased from twenty to twenty-two per cent, the plasma volume would decrease from eighty to seventy-eight per cent, a change of 2.5 per cent

which is in fact no greater than changes which might occur in plasma through haemodilution.

Fortunately, the problem is simplified in these particular experiments, since by the time the maximum changes in blood calcium or phosphate were observed, the haematocrit and haemoglobin readings had returned approximately to pre-treatment values. Therefore the changes in arterial blood calcium and inorganic phosphate probably reflect genuine alterations in the quantities of these substances in each unit volume of circulating blood.

It seems quite clear that in some respects the two main components of posterior pituitary extract, namely oxytocin and vasopressin, exerted antagonistic actions. This is seen most clearly in the haematocrit and haemoglobin readings, where the changes during the administration of posterior pituitary extract were the same as in the control experiments, but oxytocin increased the cell volume temporarily and vasopressin decreased it. A similar antagonism may have affected arterial calcium concentrations during the administration of posterior pituitary extract, where the downward trend of blood calcium concentrations seemed to be temporarily arrested.

Figure 62 shows that oxytocin administered to sheep produced a drop in arterial blood calcium level of about half a milligram per cent in half an hour and that if the animals were first sensitised with oestrogen the effect was perhaps more prolonged and severe. No effect was observed on blood inorganic phosphate until the animals were sensitised (Figure 64). These changes in arterial blood composition must be regarded as very rapid

and are comparable to the changes seen over about four hours in the venous blood of calving cows or cows developing milk fever. The problem now is to decide whether the dose rates employed produced a physiological effect which might occur in normal parturient animals.

There does not appear to be any information on the normal rate of oxytocin release by the pituitary gland of ruminants, but the oxytocic content of the jugular plasma of parturient cattle and sheep may rise to one milliunit or more per millilitre (Fitzpatrick, 1961a; Fitzpatrick and Walmsley, 1962). In a sheep this may represent an uptake from the pituitary gland of 0.5 milliunits of oxytocin per millilitre of plasma or about 100 milliunits per minute, assuming a half life for oxytocin in the circulation of forty-five to fifty seconds (Fitzpatrick, 1961a), a circulation time of the same order, a total jugular plasma flow of five per cent of the cardiac output and a total plasma volume of three litres. The calculation is, of necessity, very crude. In woman, where the peripheral oxytocin level at parturition is much lower than in sheep jugular plasma (0.02 to 0.2 milliunits per millilitre (Caldeyro-Barcia and Poseiro, 1958; Fitzpatrick, 1961 discussion), the recommended dose for elective induction of early labour is up to eight milliunits per minute (Theobald, Kelsey and Muirhead, 1956). In contrast, dose rates of eight thousand milliunits per minute for thirty minutes produced rising blood concentrations in two pregnant women near term and two women post-partum, but the final values were only between one and four milliunits of oxytocic activity per millilitre (Gonzalez-Panizza, Sica-Blanco and Mendez-Bauer, 1961). It seems, therefore, that the dose rates employed

TABLE XXVIII

The differences between the blood calcium and inorganic phosphate concentrations of sheep at the end of the hormone injections and the mean pre-treatment concentrations.

mg./100 ml.

Sheep No.	Treatment											
	Control		Posterior Pituitary		Purified Oxytocic Principle		Stilboestrol		Purified Oxytocic Principle following Stilboestrol		Vasopressin	
	Calcium	I.Phos.	Calcium	I.Phos.	Calcium	I.Phos.	Calcium	I.Phos.	Calcium	I.Phos.	Calcium	I.Phos.
31			-0.99	-0.35	-1.03	-0.29						
48	0.05	-0.09	-0.35	0.01	-0.93	0.03					0.50	0.44
77	-0.05	-0.03	0.20	-0.44	0.03	-0.96	0.30	0.31	-0.36	-1.57	-0.84?	-0.03
78	0.17	-0.42	-0.11	-0.72	-0.37	-1.07	0.30	0.21	-0.76	-1.24	1.07	-0.15

For dose rates see text.

in these experiments could have produced plasma concentrations within the normal range for parturient sheep, or could have been up to five times the natural rate of secretion for such sheep. If the dose rate was high, it would account for the changes in the arterial calcium and inorganic phosphate concentrations occurring about eight times faster than is naturally observed in, for example, the venous blood of cows developing milk fever.

It is therefore of interest to note that the effect on blood calcium and inorganic phosphate of administering posterior pituitary hormones was fairly characteristic for different animals. In sheep 31 and 48 posterior pituitary extract and purified oxytocic principle produced a more marked effect on arterial blood calcium than on inorganic phosphate levels and the converse was found in sheep 77 and 78 (Table XXVIII). Sheep 77 gave a consistently poor calcium response to oxytocin and a repeat experiment with purified oxytocic principle gave an almost identical response to that reported here. No reason can be offered for these differences, unless the responses by the sheep were influenced by the number of hours of daylight and other environmental factors, as observed by Reardon and Robinson (1961) for oestrogen.

The route of loss of calcium and phosphate from the blood in the oxytocin experiments has yet to be established. The failure to detect any reduction in the calcium content of jugular plasma of six sheep during oxytocin infusion indicates that the tissues of the head were replenishing the blood and it is therefore likely that the skeleton as a whole was acting in a similar fashion. There is no evidence from the corrected V-A data

that the calcium was lost through the alimentary canal, although losses in the saliva would not be included in this estimation. However, large salivary losses would probably have been detected in the jugular blood samples. There is, in fact, some evidence that absorption of calcium may have been improved in the non-oestrogen-sensitised animals and that it was best in those animals where arterial blood calcium levels were lowest. No changes were observed in phosphate absorption in non-sensitised sheep.

Oxytocin administered after sensitisation seemed to have a more profound effect on arterial blood calcium and inorganic phosphate levels and here there is evidence of interference with absorption. The water absorption may have been reduced a little, but it is noticeable that the calcium absorption did not improve during the oxytocin administration, as was observed in non-sensitised sheep, and the phosphate absorption was very much reduced. These changes, which have only been observed on a few animals and therefore require confirmation, suggest that oxytocin may have some action on the activity of the alimentary canal of sensitised sheep. Waring and Landgreve (1950) reviewed the literature and concluded that posterior pituitary extracts with high oxytocic acitivity cause a decrease in intestinal tone, and Lloyd (1959) observed that oxytocin exerted a constrictor effect on mesenteric blood vessels, and a pressor effect, when injected into rats in oestrus.

Vasopressin would be expected to affect the digestive tract and there is evidence of this in the reduced percentage haemoglobin difference and corrected V-A difference for inorganic phosphate during its

administration, but it did not appear to affect calcium absorption. Although vasopressin is now known to act on uterine muscle (Fitzpatrick, 1960), its exact role at parturition does not appear to have been studied. If the normal balance between oxytocin and vasopressin secretions in the pituitary is upset at the time of parturition in favour of vasopressin it could seriously affect the absorption of nutrients from the digestive tract, but there is very little evidence concerning the rate of release of these two hormones from the pituitary gland at parturition (Landgreve, Ketterer and Waring, 1955). In these experiments vasopressin tended to raise blood calcium and inorganic phosphate levels and this in itself would suggest that it does not play a very active part in the aetiology of parturient hypocalcaemia and paresis. It would also be expected to raise blood pressure, but Rubenkov (1960) found that the blood pressure of normal cows after calving and of nine cows with milk fever was lower than in the late pregnancy and after recovery.

Earlier in this thesis reference was made to the difficulty of inducing a hypocalcaemia in the venous blood of animals which are not under a calcium stress. The changes in arterial calcium values produced by oxytocin suggest that any tendency to a hypocalcaemia may be detected more readily by a routine study of arterial blood samples, rather than of jugular or mammary venous blood. The arterial blood is obviously derived from a mixture of venous bloods from various sources and it is only when samples are drawn from a vein draining an area where calcium is being lost or where any deficiency is not being corrected that a venous hypocalcaemia need be detected. In the non-pregnant,non-lactating

animal, such as the non-oestrogen-sensitised sheep injected with oxytocin, the venous drainage from the head and splanchnic regions seemed to be adequately replenished, so that at least one third of the blood returning to the heart would be of normal calcium content. This, mixing with hypocalcaemic blood from another source, would help to 'dilute' the hypocalcaemia. If, however, replenishment of the calcium content of the splanchnic blood were also impaired, as has been suggested for calving cows and perhaps also in oestrogen sensitised sheep treated with oxytocin, then this dilution would be reduced and a more severe arterial hypocalcaemia would be expected. If in addition the animal were milking, any blood transfusing the mammary gland would also return to the heart in a hypocalcaemia state and aggravate the arterial hypocalcaemia still further.

The interpretation of these findings in relation to those of the previous sections must, for the moment, remain guarded. The effect of oxytocin on the blood calcium and inorganic phosphate concentrations would probably not, by itself, precipitate parturient paresis, although it may be a contributory factor. More recent experiments with non-sensitised sheep and an infusion period of two hours indicate that the plasma calcium concentrations can rise again even during the period of infusion, so the hypocalcaemic effect may be transient.

The suggestion of interference with the efficiency of calcium and phosphate absorption in oestrogen sensitised sheep during the injection of oxytocin is, however, of potentially greater interest in view of the earlier discussion on alimentary stasis in cows at calving. Dicker and

Tyler (1953a; 1953b) have found that rats and bitches rearing large litters had lower oxytocic contents in their pituitary glands than those rearing small litters. Lactating animals also had lower oxytocic contents in their pituitaries glands than non-lactating animals, but vasopressin contents were unchanged. This was interpreted to mean that the release of oxytocin from the pituitary of lactating animals, especially those rearing large litters, was higher than normal and it could be that the oxytocin release of heavily yielding cows after calving is also high. As was shown (Section III), old cows, which are known to be more prone to milk fever, tend to have higher initial yields of milk than young cows.

Summary

Six cows were excited and stimulated by various combinations of pricking of the hindquarters, unusual noises and chasing around a yard. No changes were observed in serum calcium or blood inorganic phosphate, citric acid or glucose. Lactic and pyruvic acid concentrations in the blood were raised.

Two cows were injected with posterior pituitary extract, but no effect on rumen activity was observed.

Sheep were injected with various posterior pituitary hormones and oestrogen for thirty minutes. No changes in the calcium or haemoglobin content of jugular blood were observed during the administration of purified oxytocic principle, but arterial haemoglobin levels rose and arterial blood calcium levels fell. The effect was perhaps more marked, and arterial

inorganic phosphate levels also fell, in animals sensitised with oestrogen. The opposite effects on arterial blood were produced by vasopressin and intermediary effects by posterior pituitary extract. No effects were observed with oestrogen injections and no changes in blood magnesium levels were recorded during the injection of any of these hormones.

No change in the absorption of calcium in any of the experiments was detected by observing hepatic veno-arterial differences, except occasionally in non-oestrogen-sensitised sheep injected with oxytocin when the calcium absorption appeared to be increased. In oestrogen sensitised animals injected with purified oxytocic principle and in animals injected with vasopressin there was evidence of reduced phosphate absorption.

GENERAL DISCUSSION

Low concentrations of calcium in the blood of ruminants have been reported in association with a number of diseases, including foot and mouth disease, ketosis, alimentary dysfunction and winter scours in cattle (Seekles, 1939; Halse and Velle, 1958; Marr, 1958), Johnes disease in cattle and sheep (Stewart, McCallum and Taylor, 1945), and hypomagnesaemia and liver fluke infestation in sheep (Hughes and Kershaw, 1958; Sinclair, 1960), but the commonest clinical form in cattle is that referred to as 'milk fever' where there is no obvious aetiological factor. The majority of milk fever cases occur immediately before or within three or four days after calving, but some have been reported in association with oestrus (Messervy, 1948) and cases may occasionally occur in stock at any stage of their reproductive cycle (Nilsson, 1960) and even in male animals. Most cases respond well to simple calcium injections, but a proportion, usually occurring within twenty-four hours of calving, fail to respond readily to treatment (Marr, Moodie and Robertson, 1955).

The spectacular recoveries associated with calcium replacement therapy and the almost constant finding of hypocalcaemia in milk fever cases have led to the assumption that simple milk fever is caused by hypocalcaemia. In the writer's opinion this conclusion is not justified since there are apparently no records of typical milk fever symptoms being produced solely by the experimental lowering of serum calcium levels. Oxalate and ethylenediamine-tetraacetic acid

occasionally produce some of the symptoms, but seldom the typical recumbency associated with milk fever. Typical symptoms have been produced by administering hyoscine to an old heavily milking cow and by experimentally modifying the diet as described by Boda and Cole (1954) and Ender, Dishington and Helgebostad (1962), but these manipulations may affect the absorption of other nutrients besides calcium. These observations, together with the evidence that udder inflation may be a better treatment for parturient milk fever than calcium therapy (Marr, Moodie and Robertson, 1955; Marshak, 1956), suggest that the essential cause of the condition may be a deficiency of more than one substance in the blood.

It is even doubtful if calcium replacement therapy exerts its main effect by influencing the calcium balance of the animal, since the normal treatment which introduces only about seven grams of calcium into the circulation does not necessarily result in any permanent improvement in serum calcium levels even though complete clinical recovery may take place (Marr, Moodie and Robertson, 1955). The calcium therapy possibly acts more by having a powerful stimulatory effect on animals, perhaps by producing an acetylcholine like response in the body tissues or by sensitising the tissues to acetylcholine (Douglas and Rubin, 1961; Douglas and Poisner, 1962).

Thus hypocalcaemia is probably an essential feature of milk fever but it need not by itself produce the disease, in the same way as hypomagnesaemia is a constant feature of hypomagnesaemic tetany yet by itself need not produce the disorder. There is, in fact, no

genuine evidence that hypocalcaemia is more important to the development of clinical milk fever than the associated hypophosphataemia. The evidence that recovery from milk fever does not occur until blood phosphate levels are permanently elevated (Marr et al., 1955) may be a more important clue to the aetiology of the condition than the hypocalcaemia itself.

No theory as to the cause of parturient milk fever is complete unless it takes into consideration all the known biochemical, histological and physiological changes occurring at the time. Similarly, any theory as to the causation of parturient hypocalcaemia should take cognisance of the same background. Some of the parturient changes can now be given simple adequate explanations. For example, the changes in the circulating lymphocytes, eosinophils, neutrophils and glucose are similar in normal calving and paretic cows and probably result from the physiological stress of parturition (Merrill and Smith, 1954; Smith and Merrill, 1954). Similarly, Winquist (1959) found that the cellular changes in bone marrow were similar in normal calving and paretic cows. Variations in the degree of stress would not therefore seem to be the cause of the difference between normal and paretic cows nor between normal and severe hypocalcaemia at calving. Likewise, changes in lactic and pyruvic acid levels in the blood could be attributed to excitement alone (Section VIII).

The changes in blood citric acid at calving are more difficult to interpret because the citric acid level may be influenced by a number of physiological processes. Although citric acid does not

seem to be closely involved with calcium absorption from the intestine (Section VI), there is evidence that it may play an important part in the mobilisation of bone calcium (Neuman and Neuman, 1958). There seems to be no information on how the blood concentrations of citric acid are related to the rate of mobilisation of bone calcium, but the independence of blood citric acid and serum calcium concentrations in cows at calving and in cows injected with hyoscine, and the similar changes in citric acid levels in the blood of young and old cows at calving, suggest that further study of the citric acid levels in the peripheral blood of cows will not produce much information on the aetiology of parturient hypocalcaemia.

This only leaves plasma calcium and inorganic phosphate as the biochemical constituents of the plasma whose concentrations are known to be more severely altered in ageing animals and the investigations in Section II, III and IV make a strong case for believing that the combination of alimentary stasis at calving and high initial milk yields by old cows may predispose to the development of parturient hypocalcaemia. The roles of calcium mobilisation from the skeleton and of parathyroid hormone in the aetiology of parturient paresis were considered in the introduction (Pages 5-7). One of the actions of parathyroid hormone may be to improve the absorption of calcium from the alimentary tract, as suggested by Cramer, Suiker and Copp (1961), Todd, Fosgate, Cragle and Kamal (1962) and Sansom (1963), but this mechanism can hardly be effective if calcium and phosphate are not being moved along the alimentary tract to the sites of absorption.

In recent years there have been a number of reports concerning liver dysfunction in parturient paresis (Broberg, 1956; Osinga, 1959; Holtenius, Knudsen and Ullberg, 1962; Huhn and Lupke, 1962). Some workers have attributed these changes to intoxication arising from faulty nutrition, particularly an absolute or relative excess of protein in the diet, but Holtenius et al., (1962) related the changes they observed to gastro-intestinal disturbances. The reduced clearance of bromosulphthalein in paretic cows, considered to be evidence of liver dysfunction (Huhn and Lupke, 1962) could equally well be evidence of reduced flow of blood through the liver arising from gastro-intestinal stasis. It is, at this stage, quite clearly impossible to distinguish which, if either, is primary, alimentary stasis or liver disorder.

If the intestinal stasis is not due to temporary liver disorder an alternative explanation must be sought. Some observers tend to associate the condition of milk fever with dietary factors (Ender, Dishington and Helgebostad, 1962; Osinga, 1963), but if this were the only aetiological factor in parturient hypocalcaemia the condition might be expected to arise more frequently at other times, particularly towards peak lactation. A more likely explanation would be that some of the hormones associated with parturition and the onset of lactation could influence mineral metabolism, perhaps by affecting the activity of the alimentary tract. The fact that there are no calcium changes in the plasma of mastectomised cows at calving does not exempt those hormones necessary for parturition from being the cause

of hypocalcaemia. The hyoscine injections in Section IV presumably produced the same type of aetiological stress in each animal, but resulted in quite different biochemical pictures for calcium and phosphate depending on the age of the cows and whether or not the animals were lactating. It seems that aetiological factors tending to produce low plasma calcium will only be manifest in samples of peripheral venous blood when the loss of calcium from the circulation is sufficient to overcome normal regulatory processes. This is demonstrated in Section VIII where the administration of oxytocin to sheep produced low calcium levels in arterial blood, but apparently by the time the blood had circulated to the jugular vein any calcium deficiency was corrected. Conversely, the changes in inorganic phosphate cannot be dissociated from hormones necessary for the onset of milk secretion simply because hypophosphataemia occurs in mastectomised cows at calving. Indeed, in prepartum milked cows the plasma phosphate changes are quite different from those seen in non-prepartum milked cows (Robertson, Marr and Moodie, 1956), indicating that milking can have a depressent effect on serum phosphate levels.

The observations in Section VII indicate that a substance, which can depress plasma inorganic phosphate levels in lambs, is present in samples of jugular plasma of low inorganic phosphate content taken from cows before or at calving or from cows with milk fever. There is no evidence that this substance is one of the hormones specifically associated with parturition, since in the Queensland cows it was not

detected in those samples taken at parturition where the plasma phosphate concentrations were high. Nor is there any evidence that the substance originated from the posterior pituitary gland, since it took twelve to twenty-four hours to produce its effect in lambs, whereas injections of oxytocin and vasopressin were found to exert their effects immediately. In rats sensitised with oestrogen, the pressor activity of vasopressin is enhanced and oxytocin produces vasoconstriction of the mesenteric blood vessels and has a pressor effect (Lloyd, 1959), and in sheep there is evidence of interference with calcium and phosphate absorption under similar conditions (Section VIII). These results suggest that oxytocin could be an aetiological factor in producing alimentary stasis and hypocalcaemia in cows at calving, since the uterine responses of calving cows are those of oestrogen sensitised animals and the concentrations of oxytocin in the circulation are known to be high (Fitzpatrick, 1960; Fitzpatrick and Walmsley, 1962).

GENERAL SUMMARY

The exchange of calcium and inorganic phosphate between the blood and the tissues of cattle was briefly reviewed and an attempt made to state these exchanges in quantitative terms. It was suggested that the heavily milking cow depends on a continuous absorption of calcium from the alimentary tract and that interference with calcium absorption of even a few hours duration might lead to hypocalcaemia. To test this suggestion, the alimentary activity and milk yield of calving cows were observed daily and related to changes in the concentrations of calcium and inorganic phosphate in the serum and blood. Alimentary activity was assessed by observing feed intake, faecal output, rumen sounds and rumen movements. Also, cows were injected with sodium oxalate or hyoscine hydrobromide to study the effect of hypocalcaemia on alimentary activity or of alimentary stasis on serum calcium and blood inorganic phosphate concentrations.

Old cows at calving were found to give more milk during the first forty-eight hours of lactation than young cows and at the same time the food intake of old cows was greatly reduced. This change in food intake tended to precede any severe change in blood calcium levels. Artificially induced hypocalcaemia did not appear to affect alimentary activity but a hyoscine injection produced stasis of the alimentary tract in an old heavily milking cow, hypocalcaemia, hypophosphataemia and clinical symptoms resembling those of milk fever.

The changes in glucose and citric, lactic and pyruvic acids in the blood of calving cows were also observed, but it was concluded that these changes were not closely related to the changes in the concentrations of calcium and inorganic phosphate in the serum and blood. The absorption of calcium, magnesium, inorganic phosphate and citric acid in conscious sheep, as estimated by a portal veno-arterial technique, were also studied, with a view to developing a method for estimating the absorption of nutrients in calving cows on an hourly basis. A method of preventing hypocalcaemia by injecting secondary calcium phosphate intraperitoneally was tested in sheep.

In a search for factors which might precipitate low calcium and inorganic phosphate levels in the blood of calving cows, cow plasma was transfused into lambs and posterior pituitary hormones were administered to cattle and sheep. The plasma phosphate levels in lambs were depressed twelve to twenty-four hours after transfusing plasma of low phosphate content taken from cows before or at calving or from cows with milk fever. Administering purified oxytocic principle to sheep sensitised with oestrogen immediately depressed the arterial blood levels of calcium and inorganic phosphate and produced evidence of interference with normal absorption processes, as estimated by a hepatic veno-arterial technique.

ACKNOWLEDGMENTS

I am exceedingly grateful to my wife and to Professor A. Robertson for their constant support and encouragement throughout the course of these investigations. Without their goodwill this thesis would never have been written.

My thanks are also due to various members of the staff of the Department of Veterinary Hygiene and Preventive Medicine of the Royal (Dick) School of Veterinary Studies for technical assistance and to the Agricultural Research Council who financed some of the work. I greatly appreciate the assistance of Mr. E.V. Linford who copied the drawings and of Miss M. Fotheringham who executed the typing.

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APPENDIX I

TABLE A1

Dry Matter Feed Intake of Cows Before and After Calving

Kg/day

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	1	2	3	4	7	Mean
1. Young	1a	4.21	4.12	5.18	4.84	4.31	5.09	5.97	7.11	7.27	6.74	5.48
	1b	6.77	9.36	8.11	8.41	6.98	10.01	9.84	10.38	10.81	10.45	9.11
	2	8.76	8.96	8.74	8.36	8.70	8.69	9.07	9.16	8.76	8.87	8.81
	3a	10.10	7.74	8.82	7.68	8.68	9.76	8.74	10.32	10.52	11.52	9.39
	3b	8.54	8.18	7.78	7.77	8.12	10.28	10.04	10.49	10.65	11.02	9.29
	Mean	7.68	7.67	7.73	7.41	7.36	8.77	8.73	9.49	9.60	9.72	8.42
2. Old	4	11.70	9.91	8.40	8.87	3.93	8.10	11.09	10.85	(10.27)	(10.84)	9.39
	4M	5.73	5.03	3.52	1.44	1.27	1.39	0.34	4.21	3.16		
	5	10.51	8.00	8.54	8.39	8.51	9.62	10.50	9.22	9.41	9.30	9.20
	5MF	9.42	9.62	9.91	6.57	3.40	4.64	9.17	10.98	9.53	11.06	8.43
	8	7.61	7.66	8.83	11.14	5.42	9.79	11.49	10.27	10.70	10.99	9.39
	Mean†	9.81	8.80	8.92	8.74	5.32	8.04	10.56	10.33	9.98	10.55	9.10
1 and 2	Mean†	8.62	8.17	8.26	8.00	6.45	8.44	9.55	9.86	9.77	10.09	8.72

Analyses of Variance

Source of Variation	Young Cows d.f.	Sums of Squares	Mean Square	F	Old Cows† d.f.	Sums of Squares	Mean Square	F
Days	9	40.95	4.55	7.6***	9	90.91	10.10	4.23**
Cows	4	109.37	27.34	45.6***	3	6.30	2.10	0.88
Error	36	21.67	0.60		25	59.77	2.39	
Total	49	171.99			37	156.99		

Values in brackets are calculated.

† Excluding 4M

TABLE A2

Dry Matter Faecal Output of Cows Before and After Calving

Kg/day

Group of Cows	Cow No.	Days from Calving 4	3	2	1	0	1	2	3	4	7	Mean
1. Young	1b	2.82	2.43	2.73	2.08	1.42	3.06	3.03	3.40	3.25	3.70	2.79
	2	3.40	3.44	3.64	2.88	1.86	2.49	3.52	3.14	3.50	3.39	3.13
	3a	3.69	3.32	3.44	3.38	2.40	2.67	3.21	3.50	3.27	4.74	3.36
	Mean	3.30	3.06	3.27	2.78	1.89	2.74	3.25	3.35	3.34	3.94	3.09

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	9	7.84	0.87	6.2***
Cows	2	1.64	0.82	5.8*
Error	18	2.56	0.14	
Total	29	12.04		

TABLE A3

Milk Yield of Cows After Calving

kg. / day

Group of Cows	Cow No.	Days after Calving 0	1	2	3	4	7	Mean
1. Young	1a	(4.6)	(7.0)	8.4	9.5	11.1	12.0	8.8
	1b	9.3	10.0	8.9	10.9	13.4	14.1	11.1
	2	9.1	11.6	13.0	14.3	14.3	15.2	12.9
	3a	13.2	14.8	19.1	16.6	16.6	18.0	16.4
	3b	9.8	14.8	19.8	19.5	21.4	21.1	17.7
	Mean	9.2	11.6	13.9	14.2	15.4	16.1	13.4
2. Old	4	10.0	19.3	17.7	22.3	20.9	23.4	18.9
	4M	10.0	8.9	7.0	6.1	9.3	7.0	
	5	19.5	13.6	22.3	20.0	23.8	22.2	20.2
	5MF	9.0	13.6	16.8	16.8	18.2	21.4	16.0
	8	22.7	19.1	21.8	22.5	22.3	22.0	21.7
	Mean†	15.3	16.4	19.7	20.4	21.3	22.3	19.2
1 and 2	Mean†	11.9	13.8	16.4	16.9	18.0	18.8	16.3

Analyses of Variance

Source of Variation	Young Cows d.f.	Sums of Squares	Mean Square	F	Old Cows† d.f.	Sums of Squares	Mean Square	F
Days	5	162.7	32.53	11.74***	5	153.6	30.72	3.62*
Cows	4	328.0	82.00	29.60***	3	108.1	36.02	4.24*
Error	18	49.8	2.77		15	127.3	8.49	
Total	27	540.5			23	389.0		

Values in brackets are calculated.

† Excluding 4M.

TABLE A4

Concentrates Consumed by Cows at Calving

Kg. D.M./day

Group of Cows	Cow No.	Days from Calving									
		4	3	2	1	0	1	2	3	4	7
1. Young	1a	2.36	2.36	2.36	2.36	2.36	2.36	2.36	3.14*	3.92*	3.72
	1b	3.88	3.88	3.88	3.88	3.88	3.88	3.88	3.88	3.88	3.88
	2	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17	3.17
	3a	4.79	4.79	4.79	4.79	4.79	4.79	4.79	4.79	4.79	4.79
	3b	3.98	3.98	3.98	3.98	3.98	3.98	3.98	3.98	3.98	3.98
	Mean	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.79	3.95	3.91
2. Old	4	2.28	2.28	2.28	2.28	0.85	2.28	2.66*	3.04*	(2.85)	(3.14)
	4M	3.66	2.56	1.73	0.47	0.10	0.19	0.08[M]	2.00	1.67	
	5	3.92	3.92	3.92	3.92	3.92	3.92	3.92	3.92	3.92	3.92
	5MF	5.55	5.55	5.55	3.53	1.14[MF]	0	2.94	5.55	5.55	5.55
	8	2.88	2.35	3.76	3.91	1.96	2.19	3.91	2.71	2.96	3.82
	Mean †	3.66	3.53	3.88	3.41	1.97	2.10	3.36	3.81	3.82	4.11
1 and 2	Mean †	3.65	3.59	3.76	3.53	2.81	2.87	3.50	3.80	3.89	4.00

Values in brackets are calculated.

* Concentrate ration increased.

† Excluding 4 M.

M Clinical Metritis Developed

MF Milk Fever Developed.

TABLE A5

Hay, Roots and Silage Consumed by Cows at Calving

kg.D.M./day

Group of Cows	Cow No.	Food	Days from Calving									
			4	3	2	1	0	1	2	3	4	7
1. Young	1a	Hay	1.85	1.76	2.82	2.48	1.95	2.73	3.61	3.97	3.35	3.01
	1b	Hay	1.42	4.02	2.76	3.06	3.00	4.67	4.50	5.03	5.46	5.10
		Roots	1.47	1.47	1.47	1.47	0.11	1.47	1.47	1.47	1.47	1.47
	2	Hay	5.59	5.79	5.57	5.19	5.53	5.52	5.90	6.00	5.59	5.71
	3a	Hay	3.83	1.47	2.55	1.41	2.41	3.49	2.47	4.05	4.25	5.26
		Roots	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47
	3b	Hay	2.55	2.19	1.79	1.77	2.51	4.29	4.05	4.50	4.65	5.03
		Roots	2.02	2.02	2.02	2.02	1.64	2.02	2.02	2.02	2.02	2.02
	Mean		4.04	4.04	4.09	3.77	3.72	5.13	5.10	5.70	5.65	5.81
2. Old	4	Hay	5.70	4.78	4.23	4.41	1.84	3.86	6.44	5.52)		
		Roots	1.46	1.42	1.18	1.09	1.09	1.13	1.13	1.32)	(7.46)	(7.71)
		Silage	2.26	1.43	0.72	1.09	0.15	0.83	0.87	0.98)		
	4M	Hay	2.07	1.47	1.79	0.97	1.17	1.20	0.26[M]	2.22	1.50	
	5	Hay	4.61	2.09	2.64	2.49	2.61	3.71	4.60	3.32	3.53	3.40
		Roots	1.98	1.98	1.98	1.98	1.98	1.98	1.98	1.98	1.98	1.98
	5MF	Hay	3.86	4.07	4.35	3.03	2.26[MF]	4.64	6.23	5.42	3.98	5.51
	8	Hay	3.06	3.64	3.40	5.56	1.80	5.94	5.91	5.90	6.07	5.50
		Roots	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
	Mean†		6.15	5.27	5.04	5.33	3.35	5.94	7.21	6.53	6.18	6.44
1 & 2	Mean†		4.98	4.59	4.51	4.47	3.56	5.49	6.04	6.07	5.89	6.09

Values in brackets are calculated.

†Excluding 4M.

M Clinical Metritis Developed.

MF Milk Fever Developed.

TABLE A6

Mean Analyses of Foodstuffs Offered to Calving Cows

Cow No.	Food	Dry Matter %	Calcium g.Ca/100g.D.M.	Phosphate g.P/100g.D.M.
1a	Hay	77.5	0.66	0.20
	Concts.	86.5	0.66	0.76
1b	Hay	89.2	0.56	0.20
	Roots	10.8	0.23	0.39
	Concts.	85.8	0.93	0.97
2	Hay	82.7	0.54	0.26
	Concts.	88.0	0.57	0.81
3a	Hay	89.0	0.46	0.31
	Roots	10.8	0.23	0.39
	Concts.	88.0	0.61	0.80
3b	Hay	84.6	0.52	0.20
	Roots	11.1	0.41	0.36
	Concts.	87.5	0.52	0.67
4	Hay	81.0	0.58	0.22
	Roots	10.4	0.45	0.40
	Silage	16.6	0.71	0.33
	Concts.	83.6	0.41	0.60
4M	Hay	70.0	0.58	0.22
	Concts.	88.0	0.75	0.74
5	Hay	83.9	0.47	0.22
	Roots	10.9	0.42	0.39
	Concts.	86.3	0.43	0.77
5MF	Hay	81.5	0.46	0.16
	Concts.	87.4	0.75	0.75
8	Hay	84.7	0.58	0.23
	Roots	9.2	0.46	0.42
	Concts.	85.6	0.69	0.87

TABLE A7

Calcium Intake of Cows Before and After Calving

g.Ca/day

Group of Cows	Cow No.	Days from Calving: -4	-3	-2	-1	0	1	2	3	4	7	Mean
1. Young	1a	27.7	27.1	34.2	31.9	28.4	33.5	39.3	46.9	48.1	44.5	36.2
	1b	47.3	61.9	54.9	56.6	53.7	65.5	64.6	67.6	70.1	68.0	61.0
	2	48.9	50.1	48.9	46.7	48.6	48.6	50.7	51.2	49.0	49.6	49.2
	3a	50.2	39.3	44.3	39.0	43.6	48.6	43.9	51.2	52.2	56.8	46.9
	3b	38.3	36.8	35.1	35.0	36.6	45.8	44.8	46.7	47.4	60.2	42.7
	Mean	42.5	43.0	43.5	41.8	42.2	48.4	48.7	52.7	53.4	55.8	47.2
2. Old	4	65.0	53.6	44.3	47.6	20.1	42.7	59.5	57.4	(58.2)	(63.1)	51.2
	4M	43.8	35.2	23.4	9.1	7.5	8.4	2.1	27.9	21.2		
	5	42.3	32.6	34.7	34.1	34.6	38.9	42.3	37.3	38.1	44.5	37.9
	5MF	60.6	61.7	63.2	41.6	20.0	24.1	47.2	63.9	58.1	64.3	50.5
	8	40.3	40.7	49.6	75.8	34.6	66.1	68.3	69.6	72.6	74.7	59.2
	Mean †	52.1	47.2	48.0	49.8	27.3	43.0	54.3	57.1	56.7	61.7	49.7
1 and 2	Mean †	46.7	44.9	45.5	45.4	35.6	46.0	51.2	54.6	54.9	58.4	48.3

Analyses of Variance

Source of Variation	Young Cows d.f.	Sums of Squares	Mean Square	F	Old Cows † d.f.	Sums of Squares	Mean Square	F
Days	9	1268	140.9	7.19***	9	3317	368.5	2.80*
Cows	4	3376	844.0	43.06***	3	2318	772.6	5.87**
Error	36	706	19.6		25	3288	131.6	
Total	49	5350			37	8924		

Values in brackets are calculated.

† Excluding 4M

TABLE A8

Phosphate Intake of Cows Before and After Calving

g.P/Day

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	1	2	3	4	7	Mean
1. Young	1a	21.7	21.5	23.2	23.0	21.9	23.5	25.2	31.8	36.5	34.4	26.3
	1b	46.1	51.2	48.8	49.3	45.2	52.5	52.2	53.2	54.1	53.4	50.6
	2	37.9	38.3	37.9	37.0	37.8	37.7	38.6	38.8	37.9	38.1	38.0
	3a	56.1	48.8	51.1	48.6	51.7	55.0	51.9	56.8	57.4	60.5	53.8
	3b	36.4	36.0	35.4	35.3	35.2	39.6	39.2	40.0	40.2	46.6	38.4
	Mean	39.6	39.2	39.3	38.6	38.4	41.7	41.4	44.1	45.2	46.6	41.4
2. Old	4	39.5	34.6	30.0	31.3	14.0	29.4	37.5	38.9	(38.3)	(40.2)	33.4
	4M	31.7	29.6	16.7	5.6	3.3	4.0	1.1	19.7	15.6		
	5	49.5	43.6	44.9	44.5	44.8	47.4	49.5	46.5	47.0	44.6	46.2
	5MF	48.8	49.2	49.7	32.1	12.8	8.8	30.3	48.5	46.6	48.6	37.6
	8	35.8	34.9	47.4	59.4	30.3	44.6	60.4	49.3	52.1	58.3	47.3
	Mean†	43.4	40.6	43.0	41.8	25.5	32.6	44.4	45.8	46.0	47.9	41.1
1 and 2	Mean†	41.3	39.8	40.9	40.1	32.6	37.6	42.8	44.9	45.6	47.2	41.3

Analyses of Variance

Source of Variation	Young Cows d.f.	Sums of Squares	Mean Square	F	Old Cows † d.f.	Sums of Squares	Mean Square	F
Days	9	393	43.6	6.41***	9	1723	191.4	2.19
Cows	4	4877	1219.0	179.26***	3	1366	455.3	5.21**
Error	36	245	6.8		25	2184	87.4	
Total	49	5515			37	5273		

Values in brackets are calculated.

† Excluding 4M

TABLE A9

Percentage Dry Matter in Faeces of Three Young Cows at Calving

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	1	2	3	4	7	Mean
Young	1b	16.7	13.6	15.4	14.8	14.2	17.3	18.5	17.0	14.9	15.3	15.8
	2	14.1	14.0	13.6	13.8	13.6	13.4	14.9	13.7	13.5	14.3	13.9
	3a	14.5	16.6	14.6	13.0	17.0	15.9	17.2	16.1	14.7	14.3	15.4
	Mean	15.1	14.7	14.5	13.9	14.9	15.5	16.9	15.6	14.4	14.6	15.0

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	9	18.7483	2.0831	1.75
Cows	2	19.7627	9.8818	8.32**
Error	18	21.3907	1.1883	
Total	29	59.9017		

TABLE A10

Percentage Dry Matter in Faeces of Eleven Cows at Calving

Source of Data	Cow	Days from Calving: 3	2	1	0	1	2	3	4	Mean
Ward, Blosser & Adams (1953)	Mature Jerseys	18.1	18.3	18.0	18.3	19.7	18.5	16.6	17.4	18.1
		18.6	20.2	18.4	20.3	20.4	21.4	21.5	19.4	20.0
	Jersey Heifers	17.9	17.9	18.5	16.6	18.6	20.0	20.0	19.1	18.6
		17.6	17.2	18.4	17.9	19.4	18.9	18.5	18.7	18.3
	Mature Holsteins and Guernseys	16.3	16.1	15.7	16.8	16.9	16.6	16.2	15.2	16.2
		14.0	14.4	14.6	14.5	16.5	16.4	16.6	14.6	15.2
		16.2	17.3	15.9	15.7	21.7	18.1	17.0	16.1	17.3
		16.1	16.6	16.6	16.9	15.9	16.2	15.8	15.8	16.2
Moodie	1b	13.6	15.4	14.8	14.2	17.3	18.5	17.0	14.9	15.7
	2	14.0	13.6	13.8	13.6	13.4	14.9	13.7	13.5	13.8
	3a	16.6	14.6	13.0	17.0	15.9	17.2	16.1	14.7	15.6
	Mean	16.3	16.5	16.2	16.5	17.8	17.9	17.2	16.3	16.9

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	7	37.25	5.32	5.66***
Cows	10	259.68	25.97	27.63***
Error	70	65.87	0.94	
Total	87	362.80		

TABLE A11

Rumen Sounds Scores of Cows Before and After Calving

Group of Cows	Cow No.	4	3	2	1	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3	4	7	Mean
1. Young	1b	(2.5)	3.0	2.0	3.0	1.0	1.0	1.5	2.0	2.0	(1.8)	2.0	2.0	2.0	1.98
	2	1.5	3.0	3.0	2.0	0.0	1.5	1.5	3.0	2.0	2.5	2.5	2.5	2.0	2.08
	3a	3.0	2.5	2.5	2.5	1.5	0.0	0.5	1.0	0.5	0.0	0.0	1.0	2.0	1.31
	3b	3.0	3.0	4.0	3.0	0.0	0.0	1.0	2.5	4.0	3.0	3.0	3.0	4.0	2.58
	Mean	2.5	2.9	2.9	2.6	0.6	0.6	1.1	2.1	2.1	1.8	1.9	2.1	2.5	1.99
2. Old	4M		2.0	2.0	2.0	2.0	1.0	1.0	2.0	1.0	1.0	2.0	4.0		
	5	3.0	3.0	3.0	1.5	1.5	2.0	3.0	3.0	3.5	3.0	2.0	3.0	3.0	2.65
	5MF	4.0	4.0	3.0	2.0	1.5	0	0	3.0	2.5	2.5	3.5	3.0	2.5	2.42
	8	2.0	2.0	2.5	1.5	0.5	0.5	1.0	2.5	2.0	2.5	2.0	3.5	3.0	1.96
	Mean†	3.0	3.0	2.8	1.7	1.2	0.8	1.3	2.8	2.7	2.7	2.5	3.2	2.8	2.35
1 and 2	Mean†	2.7	2.9	2.9	2.2	0.9	0.7	1.2	2.4	2.4	2.2	2.1	2.6	2.6	2.14

Analyses of Variance

Source of Variation	Young Cows d.f.	Sums of Squares	Mean Square	F	Old Cows† d.f.	Sums of Squares	Mean Square	F
Days	12	28.24	2.35	3.65**	12	22.91	1.91	3.84**
Cows	3	10.63	3.54	5.50**	2	3.23	1.62	3.25
Error	34	21.91	0.64		24	11.94	0.50	
Total	49	60.78			38	38.08		

Values in brackets are calculated.

† Excluding 4M.

TABLE A12

Total Rumen Movements of Cows Before and After Calving

Movements / 10 min.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3	4	7	Mean
1. Young	1b	23.3	21.4	17.5	17.5	8.6	14.0	22.0	18.3	21.7	21.7	18.3	20.0	20.0	18.8
	2	17.5	24.0	21.7	22.2	17.0	18.8	22.0	15.7	22.0	22.0	16.7	20.0	18.0	19.8
	3a	18.6	20.0	16.7	20.0	20.0	23.3	16.0	18.0	20.0	20.0	20.0	22.5	18.0	19.5
2. Old	5	16.7	17.5	15.8	18.3	11.7	15.8	16.7	17.5	20.0	15.0	15.0	20.8	17.5	16.8
	8	20.0	20.0	20.0	22.0	20.0	22.2	15.0	21.7	18.3	22.5	21.8	21.7	21.7	20.5
Mean		19.2	20.6	18.3	20.0	15.5	18.8	18.3	18.2	20.4	20.2	18.4	21.0	19.0	19.1

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	12	130.58	10.88	1.69
Cows	4	105.47	26.37	4.10**
Error	48	308.71	6.43	
Total	64	544.76		

TABLE A13

Primary Rumen Movements of Cows Before and After Calving

Movements / 10 min.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3	4	7	Mean
1. Young	1b	13.3	14.3	10.0	10.0	0.0	10.0	14.0	11.7	13.3	13.3	10.0	12.5	12.0	11.1
	2	12.5	14.0	13.3	14.4	8.0	11.3	12.0	10.0	12.0	10.0	10.0	14.0	12.0	11.8
	3a	11.4	12.0	10.0	12.5	12.0	16.7	10.0	10.0	12.0	10.0	12.0	15.0	10.0	11.8
2. Old	5	10.0	10.8	9.2	11.7	6.7	10.0	9.2	10.8	10.0	9.2	10.0	10.8	10.8	9.9
	8	12.9	13.8	12.5	13.0	12.5	14.4	8.8	13.3	11.7	15.0	12.7	13.3	13.3	12.9
Mean		12.0	13.0	11.0	12.3	7.8	12.5	10.8	11.2	11.8	11.5	10.9	13.1	11.6	11.5

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	12	106.94	8.91	2.17*
Cows	4	60.32	15.08	3.67**
Error	48	197.14	4.11	
Total	64	364.40		

TABLE A14

Secondary Rumen Movements of Cows Before and After Calving

Movements / 10 min.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	⅓	⅔	1	1½	2	3	4	7	Mean
1. Young	1b	10.0	7.1	7.5	7.5	8.6	4.0	8.0	6.7	8.3	8.3	8.3	7.5	8.0	7.7
	2	5.0	10.0	8.3	7.8	9.0	7.5	10.0	5.7	10.0	12.0	6.7	6.0	6.0	8.0
	3a	7.1	8.0	6.7	7.5	8.0	6.7	6.0	8.0	8.0	10.0	8.0	7.5	8.0	7.7
2. Old	5	6.7	6.7	6.7	6.7	5.0	5.8	7.5	6.7	10.0	5.8	5.0	10.0	6.7	6.9
	8	7.1	6.2	7.5	9.0	7.5	7.8	6.3	8.3	6.7	7.5	9.1	8.3	8.3	7.7
Mean		7.2	7.6	7.3	7.7	7.6	6.4	7.6	7.1	8.6	8.7	7.4	7.9	7.4	7.6

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	12	22.24	1.85	0.85
Cows	4	9.14	2.29	1.05
Error	48	104.63	2.18	
Total	64	136.01		

TABLE A15

Serum Calcium Values of Cows Before and After Calving

mg/100 ml.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	⅓	⅔	1	1½	2	3	4	7	14	Mean
1. Young	1a	11.40	10.60	10.50	11.05	10.55	10.20	10.35	10.25	10.65	10.25	10.20	(1140)	11.30	11.10	10.70
	1b	10.00	9.95	10.00	9.90	8.85	9.20	9.15	9.25	8.80	9.50	10.08	10.50	11.20	9.45	9.63
	2	9.35	9.40	9.30	8.85	9.00	9.05	8.90	8.65	9.10	8.80	9.05	9.00	9.90	8.90	9.09
	3a	(9.31)	9.35	9.75	8.60	8.37	8.60	8.07	8.77	8.90	7.95	8.60	9.43	9.45	9.25	8.89
	3b	10.40	10.10	10.70	10.40	8.95	10.10	9.20	9.05	9.55	9.65	9.80	11.10	10.50	11.20	10.05
	Mean	10.09	9.88	10.05	9.76	9.14	9.43	9.13	9.19	9.40	9.23	9.55	10.29	10.27	9.98	9.67
2. Old	4	10.90	10.50	(11.32)	9.50	8.65	9.75	9.80	9.10	9.95	9.80	10.45	(10.40)	(10.42)	(10.73)	10.09
	4M		12.25	12.00	10.60	10.80	10.35	10.10	9.55	9.60	9.25	8.95	9.25	8.75	8.55	
	5	9.05	10.25	9.60	8.00	7.80	8.75	8.10	8.25	9.10	9.50	9.85	9.20	9.65	9.30	9.03
	5MF	10.45	10.20	10.25	9.90	8.45	5.35	4.00	6.65	7.30	7.30	8.80	8.40	9.25	9.70	8.29
	8	10.35	10.70	10.30	9.90	9.05	8.00	8.55	8.35	7.80	8.20	9.10	9.80	8.55	9.40	9.15
	Mean†	10.19	10.41	10.37	9.33	8.49	7.96	7.61	8.09	8.54	8.70	9.55	9.45	9.47	9.78	9.13
1 & 2	Mean†	10.13	10.12	10.19	9.56	8.85	8.78	8.46	8.70	9.02	9.00	9.55	9.91	9.91	9.89	9.43

Analyses of Variance

Source of Variation	Young Cows d.f.	Sums of Squares	Mean Square	F	Old Cows† d.f.	Sums of Squares	Mean Square	F
Days	13	11.7013	0.9001	6.80***	13	43.4063	3.3389	3.56***
Cows	4	30.2284	7.5571	57.12***	3	23.0454	7.6818	8.41***
Error	50	6.6162	0.1323		35	31.9883	0.9139	
Total	67	48.5459			51	98.4400		

Values in brackets are calculated.

† Excluding 4M

TABLE A16

Blood Citric Acid Values of Cows Before and After Calving

mg/100 ml.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3	4	7	14	Mean
1. Young	1b	4.30	4.10	3.77	2.47	2.47	1.23	1.40	1.02	0.99	1.06	1.53	2.02	3.40	2.12	2.28
	2	4.00	2.55	2.51	3.98	2.78	2.22	2.55	3.35	3.69	3.65	3.85	3.98	4.10	3.88	3.36
	3a	4.11	5.17	4.61	4.29	2.21	2.15	0.78	0.73	0.54	0.97	0.93	1.03	1.90	3.04	2.32
	3b	4.68	4.27	4.45	4.32	2.44	1.60	1.48	2.60	3.87	4.27	4.74	3.61	4.35	4.08	3.63
	Mean	4.27	4.02	3.84	3.77	2.48	1.80	1.55	1.93	2.27	2.49	2.76	2.66	3.44	3.28	2.90
2. Old	4M		3.95	3.28	3.32	0.55	0.05	0.00	0.00	0.00	0.52	0.19	0.59	0.46	1.00	
	5	4.43	4.55	4.55	4.51	4.02	3.56	3.48	3.36	2.89	3.80	3.91	2.78	2.38	2.75	3.64
	5MF	4.80	4.51	4.52	4.77	3.31	1.35	1.35	0.81	1.04	0.94	1.77	2.43	2.58	4.41	2.76
	8	5.11	4.89	4.85	4.89	4.89	2.26	0.98	1.36	1.17	1.97	4.17	3.24	0.72	2.04	3.04
	Mean†	4.78	4.65	4.64	4.72	4.07	2.39	1.94	1.84	1.70	2.24	3.28	2.82	1.89	3.07	3.15
1 & 2	Mean†	4.49	4.29	4.18	4.17	3.16	2.05	1.72	1.89	2.03	2.38	2.99	2.73	2.78	3.19	3.00

Analyses of Variance

Source of Variation	Young Cows d.f.	Sums of Squares	Mean Square	F	Old Cows† d.f.	Sums of Squares	Mean Square	F
Days	13	39.9870	3.0759	3.40***	13	56.5905	4.3531	5.42***
Cows	3	20.5463	6.8487	7.57***	2	5.7127	2.8563	3.56
Error	39	35.2997	0.9051		26	20.8833	0.8032	
Total	55	95.8330			41	83.1865		

† Excluding 4M

TABLE A17

Blood Glucose Values of Cows Before and After Calving

mg/100 ml.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3	4	7	14	Mean
Young	3b	50.4	44.4	61.9	55.6	79.6	45.9	55.5	44.0	41.1	32.5	29.4	26.7	36.4	27.7	45.1
	4M		49.8	54.3	52.2	68.6	60.6	49.5	43.7	36.2	37.5	40.4	36.0	26.4	33.9	
	5	53.0	60.2	65.3	73.0	72.0	49.1	40.5	40.5	47.0	45.6	44.2	44.0	24.2	25.0	48.8
Old	5MF	49.7	49.5	45.7	54.7	69.5	59.6	74.4	51.2	47.0	34.1	34.9	41.8	42.7	34.3	49.2
	8	49.8	58.5	52.6	70.4	81.3	56.5	55.6	51.1	40.9	46.7	36.5	37.5	41.5	30.2	50.9
	Mean†	50.7	53.1	56.4	63.4	75.6	52.8	56.5	46.7	44.0	39.7	36.3	37.5	36.2	29.3	48.4

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	13	8078	621.4	11.59***
Cows	3	238	79.3	1.48
Error	39	2091	53.6	
Total	55	10407		

† Excluding 4M

TABLE A18

Blood Inorganic Phosphate Values of Cows Before and After Calving

mg/100 ml.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3	4	7	14	Mean
1. Young	1a	6.53	6.71	6.42	6.09	4.04	5.04	4.67	5.13	5.22	5.10	4.54	(3.39)	4.22	3.74	5.06
	1b	5.41	5.81	6.13	5.50	3.82	5.59	5.06	4.60	3.75	3.24	3.42	2.99	4.69	4.59	4.61
	2	4.87	4.75	4.05	3.76	2.97	4.61	4.76	4.52	4.24	4.47	4.45	4.23	5.22	4.87	4.41
	3a	5.55	5.95	6.01	5.48	5.19	5.13	4.44	5.47	5.61	4.77	5.71	4.82	5.33	3.40	5.20
	3b	6.06	5.34	5.17	5.60	2.82	5.69	5.17	4.41	4.06	3.94	3.66	4.63	5.04	5.49	4.79
	Mean	5.68	5.71	5.56	5.29	3.77	5.21	4.82	4.83	4.58	4.30	4.36	4.01	4.88	4.42	4.82
2. Old	4	6.44	6.64	(6.57)	4.61	3.03	4.85	5.40	6.25	5.25	5.61	5.27	(5.64)	(4.98)	(4.99)	5.40
	4M		5.20	5.66	4.00	3.26	4.89	3.91	3.52	3.38	3.18	3.66	4.18	3.54	2.79	
	5	6.53	6.37	5.58	4.16	3.10	4.43	4.44	4.71	5.39	5.34	5.36	4.47	3.97	3.37	4.80
	5MF	5.69	5.51	5.08	5.14	3.29	1.46	0.61	3.27	3.96	5.47	5.88	5.11	3.84	2.88	4.09
	8	5.41	5.74	5.32	3.75	1.75	2.91	3.13	2.60	2.32	2.08	2.76	3.61	3.40	4.99	3.56
	Mean†	6.02	6.07	5.64	4.42	2.79	3.41	3.40	4.21	4.23	4.63	4.82	4.71	4.05	4.06	4.46
1 & 2	Mean†	5.83	5.87	5.59	4.90	3.33	4.41	4.19	4.55	4.42	4.45	4.56	4.32	4.51	4.26	4.66

Analyses of Variance

Source of Variation	Young Cows d.f.	Sums of Squares	Mean Square	F	Old Cows† d.f.	Sums of Squares	Mean Square	F
Days	13	24.6024	1.8924	3.94***	13	48.2700	3.7130	3.81***
Cows	4	5.8426	1.4606	3.04**	3	27.3062	9.1021	9.33***
Error	51	24.4951	0.4802		35	34.1509	0.9757	
Total	68	54.9401			51	109.7271		

Values in brackets are calculated.

† Excluding 4M.

TABLE A19

Blood Lactic Acid Values of Cows Before and After Calving

mg/100 ml.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3	4	7	14	Mean
Young	3b	3.35	4.33	3.21	3.16	3.31	3.10	2.25	2.30	2.78	2.41	3.31	3.33	5.75	2.27	3.20
Old	4M		3.14	4.08	3.05	7.55	5.67	5.04	4.78	3.47	4.49	3.47	3.16	4.41	5.08	
	5	3.95	3.40	2.00	3.65	5.20	2.70	2.55	2.80	2.70	2.80	2.10	3.75	2.60	(2.00)	3.01
	8	3.50	3.40	3.70	5.00	9.00	6.90	3.70	3.40	2.15	1.90	1.90	2.20	3.75	2.70	3.80
	Mean†	3.60	3.73	2.97	3.94	5.83	4.23	2.83	2.83	2.54	2.37	2.44	3.09	4.03	2.32	3.34

Analysis of Variance†

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	13	36.6649	2.8203	1.90
Cows	2	4.7516	2.3758	1.60
Error	25	37.0820	1.4832	
Total	40	78.4985		

Value in brackets is calculated.

† Excluding 4M.

TABLE A20

Blood Pyruvic Acid Values of Cows Before and After Calving

mg/100 ml.

Group of Cows	Cow No.	Days from Calving: 4	3	2	1	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3	4	7	14	Mean
Young	3b	0.77	0.85	0.79	0.77	0.74	0.62	0.53	0.51	0.50	0.55	0.91	0.94	1.08	0.93	0.75
Old	4M		0.68	0.67	0.77	1.57	1.17	1.07	1.25	1.03	1.25	0.87	1.00	1.15	1.36	
	5	0.82	0.68	0.60	0.68	1.08	0.58	0.61	0.93	0.93	0.85	0.63	1.20	0.89	(0.82)	0.81
	5MF	0.83	0.80	0.66	0.65	0.74	1.06	2.10	0.85	1.00	0.92	0.89	0.66	0.63	0.76	0.90
	8	0.72	0.79	0.97	1.01	1.29	1.24	0.66	0.52	0.50	0.48	0.53	0.59	0.93	0.78	0.79
	Mean†	0.79	0.78	0.76	0.78	0.96	0.88	0.98	0.70	0.73	0.70	0.74	0.85	0.88	0.82	0.81

Analysis of Variance†

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Days	13	0.4067	0.0312	0.37
Cows	3	0.1640	0.0546	0.64
Error	38	3.2238	0.0848	
Total	54	3.7945		

Value in brackets is calculated.

† Excluding 4M.

TABLE A21

Blood Citric Acid(x) and Serum Calcium Concentrations(y) Before and After Calving

Analysis of Covariance
Ten Cows

1. Test of significance of cow means.

Source of Variation	d.f.	Sums of Squares and Products Sx^2	Sxy	Sy^2	Errors of Estimate d.f.	Sums of Squares	Mean Square	F
Total	109	247.18	69.27	136.62	108	49.86		
Between Cows	7	72.63	-11.28	35.15				
Within Cows	102	174.55	80.55	101.47	101	64.30	0.637	
				Significance of cow means 7		14.44	2.063	3.24**

2. Individual cow regressions and significance of differences.

Cow No.	d.f.	Sums of Squares and Products Sx^2	Sxy	Sy^2	byx	Errors of Estimate d.f.	Sums of Squares
1b	13	18.38	5.35	5.50	0.291	12	3.94
2	13	6.14	0.34	0.83	0.055	12	0.81
3a	12	31.56	5.30	3.80	0.168	11	2.91
3b	13	16.35	5.35	6.41	0.327	12	4.66
4M	12	24.78	14.90	16.15	0.601	11	7.19
5	13	6.95	-0.15	7.49	-0.022	12	7.49
5MF	13	32.53	32.81	49.73	1.009	12	16.64
8	13	37.87	16.64	11.55	0.439	12	4.24
	102	174.56	80.54	101.46	0.461	94	47.88

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Deviations from average regression within cows	101	64.30		
Deviations from individual cow regressions	94	47.88	0.509	
Deviations among cow regressions	7	16.42	2.346	4.61***

Derived from Tables A15 and A16.

TABLE A22

Feed, Calcium and Phosphate Intakes, Serum Calcium and Blood Inorganic Phosphate Values for All Samples Collected from Ten Cows Before Calving

Cow No.	Days before Calving	Feed Intake kg/day	Calcium Intake g/day	Phosphate Intake g/day	Serum Calcium mg/100 ml.	Blood I.Phosphate mg/100 ml.
1a	4	4.21	27.7	21.7	11.40	6.53
	3	4.12	27.1	21.5	10.60	6.71
	2	5.18	34.2	23.2	10.50	6.42
	1	4.84	31.9	23.0	10.85	5.08
1b	10	10.86	70.0	48.1	10.00	6.19
	9	8.73	58.0	50.3	10.10	5.26
	8	9.59	62.9	52.0	10.20	6.36
	7	10.59	68.5	54.0	9.80	4.92
	6	9.55	62.6	51.0	10.40	5.92
	5	8.05	54.5	48.6	10.40	5.71
	4	6.77	47.3	46.1	10.00	5.41
	3	9.36	61.9	51.2	9.95	5.81
	2	8.11	54.9	48.8	10.00	6.13
	1	8.41	56.6	49.3	9.75	5.52
2	6	8.80	49.2	38.0	9.90	4.42
	5	8.76	48.9	37.9	9.40	5.14
	4	8.96	50.1	38.3	9.35	4.87
	3	8.74	48.9	37.9	9.40	4.75
	2	8.36	46.7	37.0	9.30	4.05
	1	8.70	48.6	37.8	8.97	3.43
3a	6	8.84	44.4	52.2	9.95	4.40
	5	10.67	52.9	58.0	9.55	5.25
	3	7.74	39.3	56.1	9.35	5.95
	2	8.82	44.3	48.8	9.75	6.01
	1	7.68	39.0	51.1	8.64	4.96
3b	4	8.54	38.3	36.4	10.40	6.06
	3	8.18	36.8	36.0	10.10	5.34
	2	7.78	35.1	35.4	10.70	5.03
	1	7.77	35.0	35.3	10.65	5.09
4	4	11.70	65.0	39.5	10.90	6.44
	3	9.91	53.6	34.6	10.50	6.64
	1	8.87	47.6	31.3	9.50	4.61
4M	3	5.03	35.2	29.6	12.25	5.20
	2	3.52	23.4	16.7	12.00	5.66
	1	1.44	9.1	5.6	10.60	4.00

TABLE A22 (cont'd)

Cow No.	Days before Calving	Feed Intake kg/day	Calcium Intake g/day	Phosphate Intake g/day	Serum Calcium mg/100 ml.	Blood I.Phosphate mg/100 ml.
5	6	8.23	33.5	44.2	10.15	4.90
	5	7.54	30.6	42.5	9.10	5.15
	4	10.51	42.3	49.5	9.05	6.16
	3	8.00	32.6	43.6	10.25	6.56
	2	8.54	34.7	44.9	9.60	5.98
	1	8.39	34.1	44.5	8.00	4.05
5MF	7	10.64	67.0	51.1	10.30	5.13
	6	9.04	58.7	48.2	10.35	5.04
	5	10.27	65.1	47.9	10.65	5.26
	4	9.42	60.6	48.8	10.45	5.69
	3	9.62	61.7	49.2	10.20	5.51
	2	9.91	63.2	49.7	10.25	5.08
	1	6.57	41.6	32.1	9.18	4.22
8	10	10.60	55.7	47.2	9.95	4.51
	9	9.46	50.6	45.2	10.40	4.37
	8	10.13	52.6	46.4	9.95	4.10
	7	8.49	46.3	43.6	10.80	4.94
	6	8.38	45.9	43.4	10.40	4.88
	5	8.33	45.6	43.3	10.65	5.33
	4	7.61	40.3	35.8	10.35	5.41
	3	7.66	40.7	34.9	10.70	5.74
	2	8.83	49.6	47.4	10.30	5.32
	1	11.14	75.8	59.4	9.77	2.74

TABLE A23

Feed Intake (x) and Serum Calcium Concentrations (y) of Ten Cows Before Calving

Analysis of Covariance*

1. Test of significance of cow means

Source of Variation	d.f.	Sums of squares and products Sx2	Sxy	Sy2	d.f.	Errors of Estimate Sums of Squares	Mean Square	F
Total	57	221.73	-28.78	28.30	56	24.57		
Between Cows	9	161.58	-34.79	17.73				
Within Cows	48	60.15	6.01	10.57	47	9.97	0.212	
Significance of cow means					9	14.60	1.622	7.64**

2. Individual cow regressions and significance of differences

Cow No.	d.f.	Sums of squares and products Sx2	Sxy	Sy2	byx	Errors of Estimate d.f.	Sums of Squares
1a	3	0.776	-0.298	0.487	-0.38	2	0.375
1b	9	13.856	-0.259	0.439	-0.02	8	0.434
2	5	0.196	0.073	0.446	0.37	4	0.419
3a	4	4.864	1.226	1.016	0.25	3	0.707
3b	3	0.407	-0.194	0.227	-0.48	2	0.134
4	2	4.098	1.906	1.040	0.47	1	0.154
4M	2	6.498	3.071	1.582	0.47	1	0.130
5	5	5.291	0.331	2.921	0.06	4	2.900
5MF	6	10.726	3.378	1.340	0.31	5	0.277
8	9	13.438	-3.218	1.074	-0.24	8	0.303
	48	60.152	6.015	10.572	0.10	38	5.834

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Deviations from average regression within cows	47	9.97		
Deviations from individual cow regressions	38	5.834	0.154	
Differences among cow regressions	9	4.136	0.460	2.99**

* Derived from Table A22

TABLE A24

Calcium Intake (x) and Serum Calcium Concentrations (y) of Ten Cows Before Calving

Analysis of Covariance[/]

1. Test of significance of cow means

Source of Variation	Sums of squares and products				Errors of Estimate			
	d.f.	Sx^2	Sxy	Sy^2	d.f.	Sums of Squares	Mean Square	F
Total	57	9961.4	-78.41	28.30	56	27.68		
Between Cows	9	7421.4	-112.45	17.73				
Within Cows	48	2540.0	34.04	10.57	47	10.12	0.215	
Significance of cow means					9	17.56	1.952	9.07**

2. Individual cow regressions and significance of differences

Cow No.	d.f.	Sums of squares and products Sx^2	Sxy	Sy^2	byx	Errors of Estimate d.f.	Sums of Squares
1a	3	34.7	-2.00	0.487	-0.058	2	0.372
1b	9	423.4	-1.56	0.439	0.004	8	0.433
2	5	6.3	0.43	0.446	0.068	4	0.418
3a	4	126.5	5.70	1.016	0.045	3	0.759
3b	3	7.3	-0.84	0.227	-0.113	2	0.132
4	2	156.2	11.64	1.040	0.075	1	0.173
4M	2	341.6	20.01	1.582	0.064	1	0.164
5	5	80.8	1.39	2.921	0.017	4	2.897
5MF	6	428.1	21.87	1.340	0.051	5	0.223
8	9	934.9	-24.61	1.074	-0.026	8	0.426
	48	2540.0	34.04	10.572	0.013	38	5.997

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Deviations from average regression within cows	47	10.12		
Deviations from individual cow regressions	38	6.00	0.158	
Differences among cow regressions	9	4.12	0.458	2.90*

[/] Derived from Table A22.

TABLE A25

Feed Intake (x) and Blood Inorganic Phosphate Concentrations (Y) of Ten Cows Before Calving

Analysis of Covariance

1. Test of Significance of Cow Means

Source of Variation	Sums of Squares and Products d.f.	Sx^2	Sxy	Sy^2	Errors of Estimate d.f.	Sums of Squares	Mean Square	F
Total	57	221.73	-8.07	38.69	56	38.40		
Between Cows	9	161.57	-9.08	14.27				
Within Cows	48	60.15	1.01	24.42	47	24.40	0.519	
Significance of Cow Means					9	14.00	1.555	2.99**

2. Individual Cow Regressions and Significance of Differences

Cow No.	d.f.	Sums of Squares and Products Sx^2	Sxy	Sy^2	byx	Errors of Estimate d.f.	Sums of Squares
1a	3	0.776	-0.516	1.671	-0.66	2	1.328
1b	9	13.856	0.700	1.834	0.05	8	1.799
2	5	0.196	0.296	1.944	1.51	4	1.495
3a	4	4.864	-0.420	1.854	-0.09	3	1.817
3b	3	0.407	0.504	0.671	1.24	2	0.047
4	2	4.098	2.311	2.503	0.56	1	1.201
4M	2	6.498	2.355	1.469	0.36	1	0.615
5	5	5.291	1.480	4.368	0.28	4	3.954
5MF	6	10.726	2.791	1.314	0.26	5	0.587
8	9	13.438	-8.492	6.792	-0.63	8	1.425
	48	60.152	1.011	24.419	0.02	38	14.268

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Deviations from average regression within cows	47	24.40		
Deviations from individual cow regressions	38	14.268	0.376	
Deviations among cow regressions	9	10.132	1.126	3.00**

Derived from Table A22.

TABLE A26

Phosphate Intake (x) and Blood Inorganic Phosphate Concentrations (y) of Ten Cows Before Calving

Analysis of Covariance

1. Test of significance of cow means

Source of Variation	d.f.	Sums of squares and products Sx^2	Sxy	Sy^2	Errors of Estimate d.f.	Sums of Squares	Mean Square	F
Total	57	6438.0	-29.12	38.69	56	38.56		
Between Cows	9	5311.9	-20.37	14.27				
Within Cows	48	1126.1	- 8.75	24.42	47	24.37	0.519	
Significance of cow means					9	14.19	1.577	3.04**

2. Individual cow regressions and significance of differences

Cow No.	d.f.	Sums of squares and products Sx^2	Sxy	Sy^2	byx	Errors of Estimate d.f.	Sums of Squares
1a	3	2.29	-1.189	1.671	-0.519	2	1.054
1b	9	45.20	-1.770	1.834	-0.039	8	1.765
2	5	0.95	0.624	1.944	0.658	4	1.533
3a	4	56.21	0.132	1.854	0.002	3	1.853
3b	3	0.81	0.685	0.671	0.849	2	0.089
4	2	34.05	6.908	2.503	0.203	1	1.102
4M	2	288.54	13.764	1.469	0.066	1	0.813
5	5	29.25	3.491	4.368	0.119	4	3.951
5MF	6	255.87	15.283	1.314	0.060	5	0.401
8	9	412.86	-46.675	6.792	-0.113	8	0.515
	48	1126.04	-8.747	24.419	-0.008	38	13.076

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Deviations from average regression within cows	47	24.37		
Deviations from individual cow regressions	38	13.076	0.344	
Deviations among cow regressions	9	11.294	1.255	3.65**

Derived from Table A22.

TABLE A27

Partial Correlations and Partial Regression Coefficients for Feed Intake (X_1), Milk Yield (X_2) and Serum Calcium Concentrations (X_3) of Ten Cows After Calving

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Serum Calcium
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	431.53	507.82	38.14
	Cows	283.32	361.29	2.04
	Within Cows	148.21	146.53	36.10
			Sx_2^2	Sx_2x_3
Milk Yield	Total		1418.42	22.45
	Cows		978.29	-46.48
	Within Cows		440.13	68.93
				Sx_3^2
Serum Calcium	Total			51.69
	Cows			27.83
	Within Cows			23.86

2. Partial correlation coefficients

	d.f.	$r_{12.3}$	$r_{13.2}$	$r_{23.1}$
Total	49	0.651**	0.261	-0.111
Cows	7	0.722	0.309	-0.409
Within Cows	40	0.281	0.365*	0.499**

3. Standard partial regression coefficients

	$b'_{21.3}$	$b'_{31.2}$	$b'_{32.1}$	$b'_{23.1}$
Total	0.672***	0.347	-0.142	
Cows	0.692*	0.408	-0.562	
Within Cows	0.261	0.329**		0.514***

4. Partial regression coefficients

	$b_{21.3}$	$b_{31.2}$	$b_{32.1}$	$b_{23.1}$
Total	1.22***	0.12	-0.03	
Cows	1.29*	0.13	-0.09	
Within Cows	0.45	0.13**		2.21***

Derived from Tables A1, A3 and A15.

TABLE A28

Partial Correlations and Partial Regression Coefficients for Calcium Intake (X_1), Milk Yield (X_2) and Serum Calcium Concentrations (X_3) of Ten Cows after Calving

1. Sums of squares and products

	Source of Variation	Calcium Intake	Milk Yield	Serum Calcium
		Sx_1^2	Sx_1x_2	Sx_1x_3
Calcium Intake	Total	15025.78	2101.49	236.22
	Cows	9764.50	1263.63	22.85
	Within Cows	5261.28	837.85	213.37
			Sx_2^2	Sx_2x_3
Milk Yield	Total		1418.42	22.45
	Cows		978.29	-46.48
	Within Cows		440.13	68.93
				Sx_3^2
Serum Calcium	Total			51.69
	Cows			27.83
	Within Cows			23.86

2. Partial correlation coefficients

	d.f.	$r_{12.3}$	$r_{13.2}$	$r_{23.1}$
Total	49	0.451**	0.259	-0.045
Cows	7	0.441	0.183	-0.329
Within Cows	40	0.286	0.356	0.498**

3. Standard partial regression coefficients

	$b'_{21.3}$	$b'_{31.2}$	$b'_{32.1}$	$b'_{23.1}$
Total	0.466***	0.290	-0.049	
Cows	0.459	0.192	-0.301	
Within Cows	0.265	0.322*		0.489**

4. Partial regression coefficients

	$b_{21.3}$	$b_{31.2}$	$b_{32.1}$	$b_{23.1}$
Total	0.143***	0.017	-0.009	
Cows	0.145	0.010	-0.051	
Within Cows	0.077	0.022*		2.098**

Derived from Tables A3, A7 and A15.

TABLE A29

Partial Correlations and Partial Regression Coefficients for Feed Intake (X_1), Milk Yield (X_2) and Blood Inorganic Phosphate Concentrations (X_3) of Ten Cows after Calving

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Blood Inorganic P.
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	431.53	507.82	52.75
	Cows	283.32	361.29	25.98
	Within Cows	148.21	146.53	26.77
			Sx_2^2	Sx_2x_3
Milk Yield	Total		1418.42	47.72
	Cows		978.29	14.39
	Within Cows		440.13	33.33
				Sx_3^2
Blood Inorganic P.	Total			46.20
	Cows			22.66
	Within Cows			23.54

2. Partial correlation coefficients

	d.f.	$r_{12.3}$	$r_{13.2}$	$r_{23.1}$
Total	49	0.635**	0.339*	-0.081
Cows	7	0.695	0.356	-0.182
Within Cows	40	0.505**	0.342	0.092

3. Standard partial regression coefficients

	$b'_{21.3}$	$b'_{31.2}$	$b'_{32.1}$	$b'_{23.1}$
Total	0.673***	0.351*	-0.098	
Cows	0.732*	0.488	-0.236	
Within Cows	0.535***	0.395*		0.084

4. Partial regression coefficients

	$b_{21.3}$	$b_{31.2}$	$b_{32.1}$	$b_{23.1}$
Total	1.218***	0.115*	-0.018	
Cows	1.360*	0.436	-0.036	
Within Cows	0.922***	0.157*		0.363

Derived from Tables A1, A3 and A18.

TABLE A30

Partial Correlations and Partial Regression Coefficients for Phosphate Intake (X_1) Milk Yield (X_2) and Blood Inorganic Phosphate Concentrations (X_3) of Ten Cows after Calving

1. Sums of squares and products

	Source of Variation	Phosphate Intake	Milk Yield	Blood Inorganic P.
		Sx_1^2	Sx_1x_2	Sx_1x_3
Phosphate Intake	Total	12215.53	2261.49	214.86
	Cows	9006.61	1652.04	90.89
	Within Cows	3208.92	609.45	123.97
			Sx_2^2	Sx_2x_3
Milk Yield	Total		1418.42	47.72
	Cows		978.29	14.39
	Within Cows		440.13	33.33
				Sx_3^2
Blood Inorganic P.	Total			46.20
	Cows			22.66
	Within Cows			23.54

2. Partial correlation coefficients

	d.f.	$r_{12.3}$	$r_{13.2}$	$r_{23.1}$
Total	49	0.521**	0.224	0.039
Cows	7	0.553	0.178	-0.018
Within Cows	40	0.414*	0.333	0.125

3. Standard partial regression coefficients

	$b'_{21.3}$	$b'_{31.2}$	$b'_{32.1}$	$b'_{23.1}$
Total	0.534***	0.262	0.044	
Cows	0.560	0.213	-0.022	
Within Cows	0.459**	0.384*		0.120

4. Partial regression coefficients

	$b_{21.3}$	$b_{31.2}$	$b_{32.1}$	$b_{23.1}$
Total	0.182***	0.016	0.008	
Cows	0.185	0.011	-0.003	
Within Cows	0.170**	0.033*		0.519

Derived from Tables A3, A8 and A18.

TABLE A31

Partial Correlations and Partial Regression Coefficients Within Cows for Feed Intake (X_1), Milk Yield (X_2) and Serum Calcium Concentrations (X_3) of Five Young Cows after Calving

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Serum Calcium
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	51.72	91.61	-0.03
	Cows	29.83	56.78	-8.02
	Within Cows	21.89	34.83	7.99
			Sx_2^2	Sx_2x_3
Milk Yield	Total		410.54	5.40
	Cows		233.74	-16.08
	Within Cows		176.80	21.48
				Sx_3^2
Serum Calcium	Total			16.41
	Cows			8.86
	Within Cows			7.55

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 19

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficient	0.306	0.437*	0.370
Standard partial regression coefficient	0.316	0.427*	0.392
Partial regression coefficient	0.900	0.251*	1.896

Derived from Tables A1, A3 and A15.

TABLE A32

Partial Correlations and Partial Regression Coefficients Within Cows for Feed Intake (X_1), Milk Yield (X_2) and Serum Calcium Concentrations (X_3) of Five Old Cows After Calving

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Serum Calcium
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	338.04	475.99	20.69
	Cows	211.72	364.30	- 7.42
	Within Cows	126.32	111.69	28.11
			Sx_2^2	Sx_2x_3
Milk Yield	Total		992.32	42.07
	Cows		658.99	- 5.37
	Within Cows		263.33	47.44
				Sx_3^2
Serum Calcium	Total			27.96
	Cows			11.65
	Within Cows			16.31

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 19

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficient	0.303	0.324	0.554**
Standard partial regression coefficient	0.266	0.283	0.558**
Partial regression coefficient	0.384	0.102	2.243**

Derived from Tables A1, A3 and A15.

TABLE A33

Partial Correlations and Partial Regression Coefficients Within Cows for Calcium Intake (X_1), Milk Yield (X_2) and Serum Calcium Concentrations (X_3) of Five Young Cows After Calving

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Serum Calcium
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	2057.53	-58.02	35.15
	Cows	1433.37	-251.67	- 7.18
	Within Cows	624.16	193.65	42.33
			Sx_2^2	Sx_2x_3
Milk Yield	Total		410.54	5.40
	Cows		233.74	-16.08
	Within Cows		176.80	21.48
				Sx_3^2
Serum Calcium	Total			16.41
	Cows			8.86
	Within Cows			7.55

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 19

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficient	0.346	0.417*	0.351
Standard partial regression coefficient	0.355	0.415*	0.368
Partial regression coefficient	0.189	0.046*	1.780

Derived from Tables A3, A7 and A15.

TABLE A34

Partial Correlations and Partial Regression Coefficients Within Cows for Calcium Intake (X_1), Milk Yield (X_2) and Serum Calcium Concentrations (X_3) of Five Old Cows After Calving

1. Sums of squares and products

	Source of Variation	Calcium Intake	Milk Yield	Serum Calcium
		Sx_1^2	Sx_1x_2	Sx_1x_3
Calcium Intake	Total	1147.61	2520.20	95.56
	Cows	6810.49	1875.99	-75.49
	Within Cows	4637.12	644.21	171.05
			Sx_2^2	Sx_2x_3
Milk Yield	Total		992.32	42.07
	Cows		658.99	- 5.37
	Within Cows		263.33	47.44
				Sx_3^2
Serum Calcium	Total			27.96
	Cows			11.65
	Within Cows			16.31

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 19

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficients	0.246	0.357	0.566*
Standard partial regression coefficients	0.217	0.303	0.587*
Partial regression coefficients	0.052	0.018	2.358*

Derived from Tables A3, A7 and A15.

TABLE A35

Partial Correlations and Partial Regression Coefficients Within Cows for Feed Intake (X_1), Milk Yield (X_2) and Blood Inorganic Phosphate Concentrations (X_3) of Five Young Cows After Calving

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Blood Inorganic P.
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	51.72	91.61	-3.14
	Cows	29.83	56.78	-0.96
	Within Cows	21.89	34.83	-2.18
			Sx_2^2	Sx^2x^3
Milk Yield	Total		410.54	13.63
	Cows		233.74	14.01
	Within Cows		176.80	-0.38
				Sx_3^2
Blood Inorganic P.	Total			11.16
	Cows			5.08
	Within Cows			6.08

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 19

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficients	0.568*	-0.221	0.118
Standard partial regression coefficients	0.579**	-0.267	0.100
Partial regression coefficients	1.645**	-0.141	0.538

Derived from Tables A1, A3 and A18.

TABLE A36

Partial Correlations and Partial Regression Coefficients Within Cows for Feed Intake (X_1), Milk Yield (X_2) and Blood Inorganic Phosphate Concentrations (X_3) of Five Old Cows After Calving

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Blood Inorganic P.
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	338.04	475.99	46.13
	Cows	211.72	364.30	17.18
	Within Cows	126.32	111.69	28.95
			Sx_2^2	Sx_2x_3
Milk Yield	Total		992.32	48.06
	Cows		658.99	14.37
	Within Cows		263.33	33.69
				Sx_3^2
Blood Inorganic P.	Total			32.76
	Cows			15.30
	Within Cows			17.46

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 19

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficients	0.477	0.455	0.193
Standard partial regression coefficients	0.493*	0.499*	0.194
Partial regression coefficients	0.712*	0.186*	0.753

Derived from Tables A1, A3 and A18.

TABLE A37

Partial Correlations and Partial Regression Coefficients Within Cows for Phosphate Intake (X_1), Milk Yield (X_2) and Blood Inorganic Phosphate Concentrations (X_3) of Five Young Cows After Calving

1. Sums of squares and products

	Source of Variation	Phosphate Intake	Milk Yield	Blood Inorganic P.
		Sx_1^2	Sx_1x_2	Sx_1x_3
Phosphate Intake	Total	2172.98	264.48	12.64
	Cows	1947.78	159.10	16.59
	Within Cows	225.20	105.38	- 3.95
			Sx_2^2	Sx_2x_3
Milk Yield	Total		410.54	13.63
	Cows		233.74	14.01
	Within Cows		176.80	- 0.38
				Sx_3^2
Blood Inorganic P.	Total			11.16
	Cows			5.08
	Within Cows			6.08

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 19

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficients	0.530*	-0.120	0.055
Standard partial regression coefficients	0.533*	-0.141	0.047
Partial regression coefficients	0.472*	-0.023	0.253

Derived from Tables A3, A8 and A18.

TABLE A38

Partial Correlations and Partial Regression Coefficients Within Cows for Phosphate Intake (X_1), Milk Yield (X_2) and Blood Inorganic Phosphate Concentrations (X_3) of Five Old Cows After Calving

1. Sums of squares and products

	Source of Variation	Phosphate Intake	Milk Yield	Blood Inorganic P.
		Sx_1^2	Sx_1x_2	Sx_1x_3
Phosphate Intake	Total	8439.71	2367.32	141.75
	Cows	5455.93	1863.25	13.83
	Within Cows	2983.78	504.07	127.92
			Sx_2^2	Sx_2x_3
Milk Yield	Total		992.32	48.06
	Cows		658.99	14.37
	Within Cows		263.33	33.69
				Sx_3^2
Blood Inorganic P.	Total			32.76
	Cows			15.30
	Within Cows			17.46

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 19

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficients	0.403	0.390	0.262
Standard partial regression coefficients	0.423	0.411	0.260
Partial regression coefficients	0.126	0.031	0.100

Derived from Tables A3, A8 and A18.

TABLE A39

Changes in the Rumen Activity of Cows Injected with Hyoscine Hydrobromide

	Cow No.	Hours from first injection										
		24	0	4	8	12	18	24	30	36	48	72
Rumen Sounds Score	F 4	2.0	1.5	0.5	0.0	0.0	0.5	1.0	1.0	1.5	1.0	1.5
	D19	1.5	3.0	0.0	0.0	1.0	0.5	2.0	0.5	3.0	3.0	2.0
	E14	2.0	2.0	0.0	0.5	0.5	0.5	0.5	1.5	2.0	2.0	2.0
	B51	2.5	2.5	0.0	0.5	0.0	1.5	1.5	0.5	2.5	2.0	3.0
	P11	3.0	3.0	0.0	0.0	0.0	0.0	3.0	2.0	2.5	2.5	3.0
	Mean	2.2	2.4	0.1	0.2	0.3	0.6	1.6	1.1	2.3	2.1	2.3
Total Rumen Movements/ 10 mins.	F 4	20	20	2	0	11	10	18	20	16	20	18
	D19	-	16	1	6	16	13	18	18	16	20	17
	E14	18	22	3	22	6	20	16	22	20	22	22
	B51	24	17	0	17	1	12	15	17	20	19	20
	P11	23	20	0	0	0	2	11	18	20	12	20
	Mean	21	19	1	9	7	11	16	19	18	18	19
Primary Rumen Movements/ 10 mins.	F 4	14	12	0	0	5	4	10	12	8	12	12
	D19	-	10	0	5	10	9	12	12	10	12	10
	E14	12	14	0	16	0	13	8	12	12	13	14
	B51	16	10	0	12	0	5	9	10	13	13	13
	P11	15	14	0	0	0	0	3	12	12	6	14
	Mean	14	12	0	7	3	6	8	12	11	11	13
Secondary Rumen Movements/ 10 mins.	F 4	6	8	2	0	6	6	8	8	8	8	6
	D19	-	6	1	1	6	4	6	6	6	8	7
	E14	6	8	3	6	6	7	8	10	8	9	8
	B51	8	7	0	5	1	7	6	7	7	6	7
	P11	8	6	0	0	0	2	8	6	8	6	6
	Mean	7	7	1	2	4	5	7	7	7	7	7

TABLE A40

Changes in the Feed, Calcium and Phosphate Intakes, Faecal Output and Milk Yield of Cows Injected with Hyoscine Hydrobromide

	Cow No.	Days from first injection 1	0	1	2	3
Feed Intake kg. D.M/day	F 4	7.7	5.3	5.4	6.6	7.9
	D19	9.1	3.4	5.4	7.1	8.2
	E14	8.9	9.5	8.1	8.6	-
	B51	14.6	11.0	1.3	5.0	12.0
	P11	13.9	7.6	11.2	14.0	13.7
	Mean	10.8	7.4	6.3	8.3	10.5
Calcium Intake g/day	F 4	27.1	13.6	14.1	20.5	27.8
	D19	25.9	11.4	15.6	20.8	23.7
	E14	49.6	51.5	44.2	46.5	-
	B51	65.4	55.4	3.5	17.9	57.6
	P11	72.0	42.4	58.7	72.9	71.2
	Mean	48.0	34.9	27.2	35.7	45.1
Phosphate Intake g/day	F 4	32.5	26.8	27.0	29.7	32.8
	D19	30.1	14.1	18.3	24.5	27.6
	E14	38.1	45.2	41.0	42.3	-
	B51	81.0	70.8	3.6	20.8	73.1
	P11	69.0	38.8	50.0	69.3	68.7
	Mean	50.1	39.1	28.0	37.3	40.4
Faecal Output kg. D.M/day	F 4	2.61	1.13	1.61	3.30	2.05
	D19	2.83	0.83	1.85	2.64	2.60
	E14	3.39	1.32	2.68	3.29	-
	B51	-	-	-	-	-
	P11	4.37	1.43	2.50	5.23	5.90
	Mean	3.30	1.18	2.16	3.62	3.52
Milk Yield kg./day	E14	15.0	12.7	15.9	15.9	15.9
	B51	14.5	7.5	5.0	6.1	6.6
	P11	21.6	3.0	7.5	12.0	15.9
	Mean	17.0	7.7	9.5	11.3	12.8

TABLE A41

Changes in the Concentrations of Blood Constituents of Cows Injected with Hyoscine Hydrobromide

Blood Constituent	Cow No.	Hours from First Injection: 24	0	4	8	12	18	24	30	36	48	72
Serum Calcium mg./100 ml.	F 4	10.00	10.40	9.50	9.90	10.10	10.35	10.15	10.50	-	10.20	10.50
	D19	10.50	10.55	10.35	9.80	10.00	9.70	10.30	10.20	10.60	10.30	10.50
	E14	9.90	8.95	8.10	8.50	8.65	8.45	9.20	8.75	9.10	8.45	-
	B51	10.45	9.90	8.57	7.70	7.30	7.70	7.50	7.70	7.40	8.10	8.83
	P11	10.80	10.60	7.50	6.60	5.40	4.90	9.30	8.80	8.10	8.25	10.40
	Mean	10.33	10.08	8.80	8.50	8.29	8.22	9.29	9.19	8.80	9.06	10.06
Serum Magnesium mg./100 ml.	F 4	2.8	2.9	2.9	2.7	2.4	2.5	2.6	2.6	2.6	3.0	2.7
	E14	2.3	2.6	2.5	2.9	2.9	2.7	2.9	2.5	2.9	2.5	2.7
	B51	2.9	2.8	2.9	3.4	3.0	3.2	3.8	3.8	3.4	3.3	3.1
	Mean	2.7	2.8	2.8	3.0	2.8	2.8	3.1	3.0	3.0	2.9	2.8
Blood Inorganic P. mg./100 ml.	F 4	4.35	4.78	3.92	4.53	4.44	3.59	4.97	5.46	4.03	4.74	4.43
	D19	4.29	4.39	3.64	3.88	2.69	2.13	2.83	4.12	4.70	4.37	4.85
	E14	5.22	5.32	3.83	2.76	2.67	2.26	4.73	5.32	5.56	4.58	4.55
	B51	5.28	4.55	4.04	4.40	3.80	3.38	3.44	3.95	3.88	4.35	5.07
	P11	5.47	5.81	4.42	4.35	4.39	3.58	4.92	5.38	5.40	4.65	3.96
	Mean	4.92	4.97	3.97	3.98	3.60	2.95	4.17	4.85	4.71	4.54	4.58
Blood Citric Acid mg./100 ml.	F 4	2.99	2.92	2.49	1.33	0.79	1.00	1.00	1.02	0.91	1.31	2.75
	D19	2.02	1.49	1.32	0.95	0.68	0.72	0.53	0.40	0.56	0.64	1.18
	E14	4.10	4.18	3.56	2.46	2.08	1.69	2.12	1.96	2.19	2.86	3.82
	B51	1.88	2.17	1.27	1.35	1.20	1.49	1.27	1.29	1.10	1.75	2.19
	P11	3.70	2.92	1.52	0.95	0.39	0.52	0.70	0.43	0.80	1.26	3.00
	Mean	2.94	2.74	2.03	1.41	1.03	1.08	1.12	1.02	1.11	1.56	2.59

TABLE A42

Partial Correlations and Partial Regression Coefficients Within Cows for Feed Intake (X_1), Milk Yield (X_2) and Serum Calcium Concentrations (X_3) of Three Milking Cows Injected with Hyoscine Hydrobromide

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Serum Calcium
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	185.95	137.77	36.426
	Cows	35.04	10.30	2.690
	Within Cows	150.91	127.47	33.736
			Sx_2^2	Sx_2x_3
Milk Yield	Total		382.47	64.748
	Cows		109.95	6.145
	Within Cows		272.52	58.603
				Sx_3^2
Serum Calcium	Total			16.6381
	Cows			0.4745
	Within Cows			16.1636

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 9

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficient	0.072	0.359	0.791
Standard partial regression coefficient	0.047	0.220	0.845
Partial regression coefficient	0.064	0.072	3.489

Derived from Tables A40 and A41.

TABLE A43

Partial Correlations and Partial Regression Coefficients Within Cows for Feed Intake (X_1), Milk Yield (X_2) and Blood Inorganic Phosphate Concentrations (X_3) of Three Milking Cows Injected with Hyoscine Hydrobromide

1. Sums of squares and products

	Source of Variation	Feed Intake	Milk Yield	Blood Inorganic P.
		Sx_1^2	Sx_1x_2	Sx_1x_3
Feed Intake	Total	185.95	137.77	12.593
	Cows	35.04	10.30	1.882
	Within Cows	150.91	127.47	10.711
			Sx_2^2	Sx_2x_3
Milk Yield	Total		382.47	16.580
	Cows		109.95	2.967
	Within Cows		272.52	13.613
				Sx_3^2
Blood Inorganic P.	Total			5.034
	Cows			0.175
	Within Cows			4.859

2. Partial correlation coefficients and partial regression coefficients within cows

d.f. = 9

	x_2x_1	x_3x_1	x_2x_3
Partial correlation coefficient	0.560	0.225	0.175
Standard partial regression coefficient	0.566	0.268	0.148
Partial regression coefficient	0.765	0.048	1.117

Derived from Tables A40 and A41.

TABLE A44

Plasma Calcium Values for Sheep Injected with Sodium Oxalate Solution for Three Hours (1g./100 lbs. l.w./hour) Following the Intraperitoneal Administration of 21.5 g. Secondary Calcium Orthophosphate in 250 ml. Water Two Days Previously

mg./100 ml.

Premedication Group	Sheep No.	Time from commencement of Oxalate infusion (hours) 0	1	2	3
1. Control No premedication	23	9.12	8.35	7.35	5.52
	50	9.43	9.08	7.72	5.35
	56	10.20	9.35	7.80	3.00
	100	9.22	8.95	6.30	4.40
	Mean	9.49	8.93	7.29	4.57
2. $CaHPO_4.2H_2O$ Not washed, Not heated	23	9.70	8.56	7.20	5.22
	101	8.50	6.35	5.85	4.17
	Mean	9.10	7.46	6.53	4.70
3. $CaHPO_4.2H_2O$ Washed thoroughly, Not heated	60	9.30	8.55	6.80	6.65
	101	9.51	8.82	6.86	6.74
	Mean	9.41	8.69	6.83	6.70
4. $CaHPO_4.2H_2O$ Boiled 15 mins. Not washed	66	9.00	8.95	7.35	7.00
	100	9.00	8.78	7.89	6.85
	Mean	9.00	8.87	7.62	6.93
5. $CaHPO_4.2H_2O$ Boiled 15 mins. and washed	102	7.88	7.64	5.39	4.67
	103	7.94	7.92	5.84	4.85
	Mean	7.91	7.78	5.62	4.76

TABLE A45

Plasma Inorganic Phosphate Values for Sheep Injected with Sodium Oxalate Solution for Three Hours (1g./100 lbs. l.w./hour) Following the Intraperitoneal Administration of 21.5 g. Secondary Calcium Orthophosphate in 250 ml. Water Two Days Previously

mg./100 ml.

Premedication Group	Sheep No.	Time from commencement of Oxalate infusion (hours) 0	1	2	3
1. Control No premedication	23	2.29	2.00	1.53	1.13
	50	3.26	2.09	2.03	1.54
	56	4.23	3.94	2.84	4.10
	100	4.92	4.66	4.84	3.84
	Mean	3.68	3.17	2.81	2.65
2. $CaHPO_4.2H_2O$ Not washed, Not boiled	23	3.38	3.16	3.04	2.38
	101	4.65	4.83	4.40	4.38
	Mean	4.02	4.00	3.72	3.38
3. $CaHPO_4.2H_2O$ Washed thoroughly, Not heated	60	5.30	6.30	6.60	5.16
	101	3.63	4.24	3.41	2.87
	Mean	4.47	5.27	5.01	4.02
4. $CaHPO_4.2H_2O$ Boiled 15 mins. Not washed	66	5.64	5.32	5.80	4.42
	100	3.80	3.88	3.26	3.25
	Mean	4.72	4.60	4.58	3.84
5. $CaHPO_4.2H_2O$ Boiled 15 mins. and washed	102	6.05	5.70	5.04	5.04
	103	7.06	6.05	6.24	5.74
	Mean	6.56	5.88	5.64	5.39

TABLE A46

Analyses of Variance on Calcium Data of Sheep Injected with Sodium Oxalate

Source of Variation	d.f.	Sums of Squares	Mean Square	F
1. Groups 1 and 3				
Groups	1	0.5896	0.5896	2.43
Animals within Groups	4	0.9715	0.2428	
Animals	5	1.5611		
Time	3	63.7114	21.2371	44.10***
Groups x Time	3	5.8225	1.9408	4.03*
Remainder	12	5.7794	0.4816	
Total	23	76.8744		
2. Groups 1 and 4				
Groups	1	1.5052	1.5052	6.49
Animals within Groups	4	0.9277	0.2319	
Animals	5	2.4329		
Time	3	58.2125	19.4042	39.27***
Groups x Time	3	6.3777	2.1159	4.28*
Remainder	12	5.9303	0.4941	
Total	23	72.9534		
3. Group 1 V. Groups 2 and 3				
Groups	1	1.4921	1.4921	7.89*
Animals within Groups	6	1.1353	0.1892	
Animals	7	2.6274		
Time	3	66.0385	22.0128	59.49***
Groups x Time	3	8.7925	2.9308	7.92**
Remainder	18	6.6605	0.3700	
Total	31	84.1189		

From Table A44.

TABLE A47

Plasma calcium, magnesium, inorganic phosphate, citric acid and whole blood chloride values and haemoglobin readings for 55 pairs of carotid arterial and portal venous samples of blood from seven castrated male sheep, together with arterial haematocrit readings.

Sheep No.	Sample No.	Plasma calcium*		Plasma magnesium*		Plasma inorganic phosphate*		Plasma citric acid*		Whole blood chloride*		Hb. reading†		Hæmatocrit‡
		A	V	A	V	A	V	A	V	A	V	A	V	A
M1	1	10·60	10·70	1·95	2·07	7·30	7·53	0·90	0·75	..	..	1·311	1·279	27·0
	2	10·45	10·60	1·95	2·13	6·27	6·48	2·95	2·80	..	..	1·078	1·080	23·0
	3	10·50	10·50	2·32	2·44	5·63	5·93	3·40	4·00	..	..	1·056	1·025	22·8
	4	11·00	11·00	2·62	2·92	6·04	6·11	3·60	3·60	..	..	0·994	1·103	21·3
	5	10·70	10·70	2·34	2·29	5·54	5·65	4·10	4·70	..	..	0·871	0·829	18·3
	6	10·35	10·25	2·07	2·13	5·41	5·85	2·30	2·40	..	..	0·872	0·783	21·0
	7	11·00	10·75	2·11	2·15	4·41	4·76	3·00	3·25	..	..	0·870	0·830	17·8
	8	10·20	9·80	2·54	2·54	6·19	6·39	5·00	5·20	..	..	0·700	0·687	16·0
	9	9·80	9·45	2·05	1·98	4·87	4·98	2·40	2·40	..	..	0·767	0·732	17·5
	10	10·40	10·40	1·91	2·03	4·02	4·07	4·40	4·40	..	..	0·742	0·706	15·0
	11	11·15	11·10	2·46	2·46	6·52	6·36	5·58	6·12	..	..	0·700	0·712	16·0
	12	10·30	10·50	1·52	1·62	5·98	5·98	5·92	6·10	..	..	0·819	0·847	18·0
M2	1	10·60	10·30	1·92	1·88	6·48	6·61	0·60	0·65	..	..	1·410	1·322	29·0
	2	11·30	11·50	2·03	1·97	7·04	6·78	0·55	0·65	..	..	1·166	1·074	27·0
	3	9·90	9·58	2·03	2·21	5·84	5·93	0·30	0·50	..	..	0·821	0·772	18·5
	4	8·50	8·30	2·63	2·61	5·07	5·38	..	..	..	..	0·726	0·723	15·0
	5	8·83	8·40	2·36	2·47	6·75	7·07	1·40	1·35	..	..	0·894	0·884	21·0
	6	7·53	7·45	2·58	2·69	8·33	8·56	2·55	2·30	..	..	0·914	0·836	21·0
	7	9·40	9·20	2·78	2·76	5·81	6·13	3·90	4·07	..	..	0·885	0·838	20·3
	8	8·50	8·10	1·70	2·17	5·72	5·98	1·35	1·45	..	..	0·967	0·900	21·8
	9	9·15	9·00	2·36	2·50	5·61	5·60	0·75	0·90	..	..	0·963	0·904	20·5
	10	9·85	9·20	2·62	2·47	6·39	6·43	0·80	1·10	..	..	0·874	0·850	18·3
	11	9·50	9·60	1·54	1·52	6·35	6·47	1·85	2·00	..	..	0·798	0·764	18·0
	12	9·60	9·40	1·99	2·20	5·75	6·18	2·19	2·35	..	..	1·065	1·022	23·0
	13	9·60	9·75	2·22	2·38	7·43	7·74	1·40	1·95	..	..	1·038	0·956	22·5
	14	9·68	9·70	2·18	2·36	8·44	8·84	2·75	2·95	..	..	1·000	0·970	21·5
	15	9·10	8·97	2·33	2·20	4·46	5·00	3·75	3·60	..	..	0·918	0·900	19·5
	16	9·10	9·40	2·42	2·58	6·11	6·68	4·00	4·20	..	..	1·056	1·050	23·0
	17	8·63	8·55	2·24	2·46	6·33	6·36	3·15	3·60	..	..	1·072	1·067	23·0
	18	9·20	9·23	2·28	2·28	6·35	6·91	3·28	3·85	..	..	1·235	1·138	26·0
M3	1	11·40	11·26	2·11	2·15	5·50	5·35	1·20	1·10	296	297	1·554	1·498	..
M4	1	10·83	10·83	1·83	1·80	5·68	5·72	0·98	1·38	268	267	1·530	1·367	33·0
	2	10·70	10·68	1·99	1·97	5·33	5·38	1·33	1·39	321	321	1·289	1·362	25·8
	3	10·88	10·88	1·90	1·83	3·68	3·68	1·87	1·87	332	341	1·152	1·088	24·0
	4	10·39	10·41	2·70	2·48	3·64	3·81	1·94	2·10	352	341	0·924	0·872	20·0
M5	1	10·56	10·55	2·22	2·22	7·65	7·98	3·53	3·03	317	316	1·726	1·656	38·0
	2	10·37	10·30	1·92	1·66	5·50	5·65	4·45	4·82	316	316	1·284	1·262	28·0
	3	10·37	10·33	2·01	2·11	8·44	8·66	3·79	3·68	301	299	1·279	1·244	27·0
	4	9·30	9·52	2·32	2·36	9·18	9·84	3·47	4·43	331	325	1·385	1·385	30·0
	5	10·36	10·28	1·86	1·93	7·70	7·87	3·36	3·75	321	319	1·215	1·216	25·0
	6	9·09	9·12	2·11	1·98	7·44	7·11	2·57	3·59	328	320	1·122	1·112	24·0
	7	..	..	..	..	..	..	..	..	352	340	1·045	0·998	23·0
	8	10·32	10·36	2·59	2·72	8·22	8·73	3·48	3·80	317	310	0·934	0·931	20·0
M6	1	9·73	9·69	2·36	2·59	7·51	7·75	1·24	1·31	309	318	1·420	1·388	30·0
	2	10·14	10·03	2·16	2·24	6·83	7·00	2·35	2·81	315	313	1·346	1·315	28·5
	3	10·50	10·38	2·36	2·30	6·57	6·98	2·20	2·00	341	344	1·215	1·112	26·0
	4	9·85	9·82	2·44	2·43	6·28	6·70	2·50	2·47	326	326	1·184	1·174	24·5
	5	10·04	9·90	2·37	2·38	5·25	5·90	0·93	0·93	356	357	1·049	0·951	22·5
	6	9·08	9·42	2·34	2·28	4·26	4·68	0·46	0·72	342	345	1·062	1·016	22·0
M7	1	10·05	9·92	2·39	2·34	7·40	7·38	2·40	2·50	328	338	1·426	1·246	30·0
	2	10·42	10·23	2·41	2·46	5·79	6·04	0·29	0·92	343	341	1·237	1·211	25·0
	3	9·20	9·00	2·52	2·48	6·92	7·50	0·96	0·91	343	342	1·194	1·194	24·0
	4	9·75	10·05	2·36	2·46	7·51	7·94	1·08	1·62	327	330	1·156	1·137	24·0
	5	9·49	9·65	2·38	2·45	5·96	6·04	1·13	1·72	332	334	1·199	1·087	25·5
	6	9·78	9·81	2·23	2·29	5·45	5·43	1·71	1·75	334	333	1·123	1·134	24·0

* mg./100 ml. plasma or blood.

† Uvispek readings.

‡ 3000 g. for 30 min. in 1.5 mm. bore haematocrit tubes.

TABLE A48

Plasma calcium, magnesium, inorganic phosphate, citric acid and whole blood chloride values and haemoglobin readings for 62 pairs of carotid arterial and portal venous samples of blood from nine female sheep, together with arterial haematocrit readings.

Sheep No.	Sample No.	Plasma calcium*		Plasma magnesium*		Plasma inorganic phosphate*		Plasma citric acid*		Whole blood chloride*		Hb. reading†		Hæmatocrit‡
		A	V	A	V	A	V	A	V	A	V	A	V	A
F1	1	11·10	11·00	2·40	2·20	4·90	5·28	1·05	1·45	..	..	0·588	0·557	..
	2	12·40	11·60	2·56	2·61	7·76	7·45	0·70	0·95	..	..	0·650	0·602	..
	3	9·80	9·55	2·58	2·52	7·16	7·08	0·70	1·10	..	..	0·647	0·646	..
	4	8·85	8·40	2·41	2·45	6·17	6·29	0·85	0·75	..	..	0·645	0·601	..
F2	1	10·58	10·98	2·97	3·03	4·12	4·31	0·75	1·30	..	..	1·212	1·150	..
	2	10·50	10·15	2·41	2·30	5·28	5·76	0·75	0·75	..	..	1·096	1·058	..
	3	9·53	9·43	3·09	2·99	5·77	6·02	0·65	0·75	..	..	0·984	0·966	..
	4	10·10	10·00	2·32	2·32	4·25	4·27	0·30	0·35	..	..	0·879	0·819	18·0
	5	10·00	9·80	2·30	2·43	4·68	5·04	0·80	0·85	..	..	0·877	0·847	18·3
	6	9·48	9·35	1·73	1·72	5·50	5·27	0·30	0·60	..	..	0·872	0·866	18·0
F3	1	10·07	9·80	1·64	1·73	5·75	6·25	0·30	0·70	..	..	1·113	1·030	24·0
	2	8·88	8·78	1·64	1·74	4·80	4·50	0·00	1·00	..	..	1·116	1·000	22·5
	3	8·89	8·91	1·34	1·40	5·20	5·80	0·60	1·41	..	..	0·993	1·030	18·0
F4	1	9·91	10·10	1·64	1·65	5·36	5·10	0·45	0·70	..	..	1·553	1·335	..
	2	9·23	9·23	1·57	1·57	5·00	5·00	0·90	0·60	..	..	1·243	1·062	26·5
F5	1	12·10	11·81	1·96	1·79	8·40	8·45	1·34	1·71	302	311	1·511	1·364	33·7
	2	10·92	10·70	1·76	1·85	5·72	5·72	1·11	1·73	331	327	1·286	1·122	27·2
	3	9·63	9·70	2·36	2·40	5·84	5·80	0·92	1·30	326	321	1·249	1·127	26·0
	4	..	..	..	..	..	..	..	..	302	304	1·158	1·129	24·5
F6	1	10·82	10·66	2·64	2·50	6·96	6·98	0·62	0·92	315	318	1·476	1·500	32·5
	2	..	..	..	..	..	..	..	..	337	331	1·023	1·034	27·0
	3	10·50	10·38	2·33	2·36	4·33	4·35	3·21	3·32	315	318	1·304	1·290	28·4
	4	10·19	10·19	2·20	1·95	3·51	3·53	4·45	5·90	331	331	1·058	1·111	23·0
	5	9·90	9·84	2·44	2·32	5·02	4·81	3·44	4·63	347	342	1·083	1·085	25·0
	6	10·20	10·24	2·03	1·93	4·19	4·35	2·09	2·27	314	318	1·123	1·104	25·5
	7	9·56	9·56	2·56	2·66	4·92	5·16	0·48	0·82	334	334	1·087	1·077	24·5
	8	9·13	9·19	2·04	1·99	5·27	5·45	1·05	1·46	353	354	1·126	1·136	25·5
	9	10·44	10·44	2·15	2·15	5·28	5·47	1·02	1·30	354	347	0·992	1·026	23·0
	10	10·95	10·78	1·67	1·81	4·61	5·00	..	..	336	336	0·997	1·022	23·0
	11	10·89	10·90	1·85	1·85	4·11	4·53	1·25	1·35	334	334	1·086	1·041	24·5
	12	11·15	11·10	2·52	2·52	4·93	5·30	1·50	1·28	272	287	1·151	1·156	25·0
	13	11·73	11·92	2·36	2·36	3·75	3·83	5·55	6·71	257	268	1·206	1·152	25·0
	14	9·58	9·75	1·57	1·50	6·31	7·14	4·10	4·60	316	313	1·622	1·617	36·0
	15	10·68	10·66	2·38	2·19	5·77	5·83	4·46	4·96	303	309	1·477	1·401	33·0
	16	10·79	10·92	2·30	2·34	5·02	5·39	2·53	3·32	322	326	1·295	1·293	30·0
	17	10·85	10·76	2·20	2·16	4·16	4·58	4·65	4·81	337	332	1·201	1·265	27·0
F7	1	10·81	10·77	1·88	1·87	7·90	8·04	3·13	3·65	328	325	1·644	1·420	35·0
	2	10·80	10·79	1·73	1·78	5·58	5·08	1·18	1·64	330	337	1·625	1·415	35·0
	3	9·95	9·98	1·85	1·96	6·09	6·40	2·50	2·26	320	326	1·303	1·217	27·0
	4	10·77	10·62	2·20	2·20	6·75	6·92	3·78	4·15	313	316	1·776	1·665	37·0
	5	10·39	10·64	2·45	2·40	5·41	5·41	4·02	4·22	320	310	1·869	1·732	38·0
	6	9·88	9·60	2·09	2·09	4·38	4·45	0·47	0·98	331	332	1·503	1·366	30·0
	7	10·00	10·10	1·86	1·90	3·98	3·79	1·16	1·14	316	319	1·354	1·327	28·0
	8	10·09	10·19	1·56	1·67	2·87	2·93	0·69	1·00	333	340	1·262	1·093	25·5
	9	11·09	11·10	1·85	1·98	6·20	6·24	0·75	0·98	329	330	1·352	1·224	28·0
	10	10·98	10·59	2·27	2·26	5·38	6·10	3·46	4·04	333	333	1·508	1·412	31·0
	11	10·43	10·51	1·62	1·67	3·13	3·05	0·80	1·22	385	392	1·249	1·028	26·0
	12	11·18	11·05	1·65	1·63	3·39	3·22	0·26	0·52	393	388	0·985	0·924	20·0
	13	10·20	10·20	1·56	1·61	6·33	6·75	0·52	0·67	321	327	1·327	1·163	26·5
	14	10·72	10·52	1·63	1·62	5·64	5·71	0·33	0·67	310	315	1·517	1·325	29·5
	15	9·96	10·35	1·18	1·20	3·61	3·52	0·26	0·61	346	341	0·989	0·932	20·0
	16	9·92	9·97	1·16	1·24	3·00	3·20	0·20	0·35	337	340	0·932	0·895	19·0
F8	1	11·21	11·45	1·91	1·91	8·90	8·72	2·96	3·41	345	350	1·570	1·365	32·5
	2	10·70	10·80	1·91	1·92	7·26	7·31	2·74	2·63	338	338	1·237	1·228	26·0
	3	10·91	10·83	1·94	2·03	5·33	5·48	4·54	4·71	323	344	1·247	1·260	25·0
	4	9·88	9·97	1·89	1·85	4·51	4·35	3·05	2·95	316	333	1·239	1·132	26·0
	5	10·60	10·72	2·02	2·06	6·18	6·36	4·55	4·80	323	326	1·325	1·273	26·0
	6	10·60	10·60	1·91	1·91	6·41	6·73	4·17	5·00	315	313	1·034	1·055	21·0
	7	10·70	10·67	1·93	1·89	5·23	5·88	4·43	4·76	315	319	1·236	1·157	25·0
	8	11·06	11·03	1·99	2·06	4·90	4·62	4·48	4·52	334	336	1·235	1·088	25·0
F9	1	10·19	10·27	2·50	2·50	6·53	6·62	4·40	4·80	299	302	1·811	1·623	39·0
	2	9·88	9·79	2·01	1·95	5·12	5·00	5·49	5·61	303	305	1·617	1·445	35·0

* mg./100 ml. plasma or blood.

† Uvispek readings.

‡ 3000 g. for 30 min. in 1.5 mm. bore tubes.

TABLE A49

Portal Veno-Arterial Differences and Percentage Haemoglobin Differences of All Samples Collected from Castrated Male Sheep

mg./100 ml.

Sheep No.	Sample No.	Plasma Calcium		Plasma Magnesium		Plasma Inorganic P.		Plasma Citric Acid		Percentage Haemoglobin Difference
		A*	C*	A*	C*	A*	C*	A*	C*	
M1	1	0.10	0.37	0.12	0.17	0.23	0.42	-0.15	-0.13	2.5
	2	0.15	0.13	0.18	0.18	0.21	0.20	-0.15	-0.16	-0.2
	3	0.00	0.31	0.12	0.19	0.30	0.48	0.60	0.72	3.0
	4	0.00	-1.09	0.30	0.01	0.07	-0.53	0.00	-0.36	-9.9
	5	0.00	0.55	-0.05	0.07	0.11	0.40	0.60	0.84	5.1
	6	-0.10	1.07	0.06	0.30	0.40	1.11	0.10	0.37	11.4
	7	-0.25	0.27	0.04	0.14	0.35	0.58	0.25	0.41	4.8
	8	-0.40	-0.21	0.00	0.05	0.20	0.32	0.20	0.30	1.9
	9	-0.35	0.10	-0.07	0.03	0.11	0.35	0.00	0.12	4.8
	10	0.00	0.53	0.12	0.22	0.05	0.26	0.00	0.22	5.1
	11	-0.05	-0.24	0.00	-0.04	-0.16	-0.27	0.54	0.44	-1.7
	12	0.20	-0.15	0.10	0.05	0.00	-0.20	0.18	-0.02	-3.3
M2	1	-0.30	0.39	-0.04	0.09	0.13	0.57	0.05	0.09	6.7
	2	0.20	1.19	-0.06	0.11	-0.26	0.32	0.10	0.16	8.6
	3	-0.32	0.28	0.18	0.32	0.09	0.46	0.20	0.23	6.3
	4	-0.20	-0.17	-0.02	-0.01	0.31	0.33	-	-	0.4
	5	-0.43	-0.34	0.11	0.14	0.32	0.40	-0.05	-0.04	1.1
	6	-0.08	0.61	0.11	0.36	0.23	1.03	-0.25	-0.04	9.3
	7	-0.20	0.32	-0.02	0.13	0.32	0.66	0.17	0.40	5.6
	8	-0.40	0.20	0.47	0.63	0.26	0.70	0.10	0.21	7.4
	9	-0.15	0.44	0.14	0.30	-0.01	0.35	0.15	0.21	6.5
	10	-0.65	-0.39	-0.15	-0.08	0.04	0.22	0.30	0.33	2.8
	11	0.10	0.53	-0.02	0.05	0.12	0.36	0.15	0.24	4.5
	12	-0.20	0.19	0.21	0.30	0.43	0.69	0.16	0.26	4.2
	13	0.15	0.99	0.16	0.36	0.03	0.98	0.55	0.72	8.6
	14	0.02	0.32	0.18	0.25	0.40	0.67	0.20	0.29	3.1
	15	-0.13	0.05	-0.13	-0.09	0.54	0.64	-0.15	-0.08	2.0
	16	0.30	0.36	0.16	0.18	0.57	0.61	0.20	0.23	0.6
	17	-0.08	-0.04	0.22	0.23	0.03	0.06	0.45	0.47	0.5
	18	0.03	0.81	0.00	0.19	0.56	1.15	0.57	0.90	8.5
M3	1	-0.14	0.28	0.04	0.12	-0.15	0.05	-0.10	0.12	3.7
M4	1	0.00	1.29	-0.03	0.18	0.04	0.72	0.40	0.56	11.9
	2	-0.02	-0.60	-0.02	-0.13	0.05	-0.24	0.06	-0.02	-5.4
	3	0.00	0.64	-0.07	0.04	0.00	0.22	0.00	0.11	5.9
	4	0.02	0.64	-0.22	-0.07	0.17	0.40	0.16	0.29	6.0
M5/										

TABLE A49 (Cont'd)

Sheep No.	Sample No.	Plasma Calcium		Plasma Magnesium		Plasma Inorganic P.		Plasma Citric Acid		Percentage Haemoglobin Difference
		A*	C*	A*	C*	A*	C*	A*	C*	
M5	1	-0.01	0.43	0.00	0.09	0.33	0.67	-0.50	-0.37	4.2
	2	-0.07	0.11	-0.26	-0.23	0.15	0.25	0.37	0.45	1.7
	3	-0.04	0.25	0.10	0.16	0.22	0.46	-0.11	-0.01	2.8
	4	0.22	0.22	0.04	0.04	0.66	0.66	0.96	0.96	0.0
	5	-0.08	-0.09	0.07	0.07	0.17	0.16	0.39	0.39	-0.1
	6	0.03	0.11	-0.13	-0.11	-0.33	-0.27	1.02	1.05	0.9
	7	-	-	-	-	-	-	-	-	4.7
	8	0.04	0.07	0.13	0.14	0.51	0.54	0.32	0.33	0.3
M6	1	-0.04	0.18	0.23	0.29	0.24	0.42	0.07	0.10	2.3
	2	-0.11	0.12	0.08	0.13	0.17	0.33	0.46	0.52	2.3
	3	0.12	0.85	-0.06	0.15	0.41	1.06	-0.20	-0.01	9.3
	4	-0.03	0.06	-0.01	0.01	0.42	0.48	-0.03	-0.01	0.9
	5	-0.14	0.88	0.01	0.26	0.65	1.26	0.00	0.10	10.3
	6	0.34	0.76	-0.06	0.04	0.42	0.63	0.26	0.29	4.5
M7	1	-0.13	1.30	-0.05	0.29	-0.02	1.04	0.10	0.46	14.4
	2	-0.19	0.02	0.05	0.10	0.25	0.38	0.63	0.65	2.1
	3	-0.20	-0.20	-0.04	-0.04	0.58	0.58	-0.05	-0.05	0.0
	4	0.30	0.47	0.10	0.14	0.43	0.56	0.54	0.57	1.7
	5	0.16	1.15	0.07	0.32	0.08	0.70	0.59	0.77	10.3
	6	0.03	-0.07	0.06	0.04	-0.02	-0.07	0.04	0.02	-1.0

* A - Apparent C - Corrected

Data derived from Table A47.

TABLE A50

Portal Veno-Arterial Differences and Percentage Haemoglobin Differences of All Samples Collected from Female Sheep

mg./100 ml.

Sheep No.	Sample No.	Plasma Calcium		Plasma Magnesium		Plasma Inorganic P.		Plasma Citric Acid		Percentage Haemoglobin Difference
		A*	C*	A*	C*	A*	C*	A*	C*	
F1	1	-0.10	0.52	-0.20	-0.08	0.38	0.68	0.40	0.48	5.6
	2	-0.80	0.13	0.05	0.26	-0.31	0.29	0.25	0.33	8.0
	3	-0.25	-0.25	-0.06	-0.06	-0.08	-0.08	0.40	0.40	0
	4	-0.45	0.16	0.04	0.22	0.12	0.58	-0.10	-0.04	7.3
F2	1	0.40	0.99	0.06	0.22	0.19	0.42	0.55	0.62	5.4
	2	-0.35	0.02	-0.11	-0.03	0.48	0.69	0.00	0.03	3.6
	3	-0.10	0.08	-0.10	-0.04	0.25	0.36	0.10	0.11	1.9
	4	-0.10	0.63	0.00	0.17	0.02	0.33	0.05	0.08	7.3
	5	-0.20	0.14	0.13	0.22	0.36	0.54	0.05	0.08	8.5
	6	-0.13	-0.06	-0.01	0.00	-0.23	-0.19	0.30	0.30	0.7
F3	1	-0.27	0.52	0.09	0.23	0.50	1.01	0.40	0.46	8.1
	2	-0.10	0.92	0.10	0.30	-0.30	0.22	1.00	1.12	11.6
	3	0.02	-0.30	0.06	0.01	0.60	0.39	0.81	0.76	-3.6
F4	1	0.19	1.84	0.01	0.28	-0.26	0.57	0.25	0.36	16.3
	2	0.00	1.57	0.00	0.27	0.00	0.85	-0.30	-0.20	17.0
F5	1	-0.29	0.98	-0.17	0.02	0.05	0.96	0.37	0.55	10.8
	2	-0.22	1.34	0.09	0.36	0.00	0.84	0.62	0.87	14.6
	3	0.07	1.12	0.04	0.30	-0.04	0.58	0.38	0.52	10.8
	4	-	-	-	-	-	-	-	-	2.6
F6	1	-0.16	-0.33	-0.14	-0.18	0.02	-0.09	0.30	0.29	-1.6
	2	-	-	-	-	-	-	-	-	-1.1
	3	-0.12	-0.01	0.03	0.06	0.02	0.07	0.11	0.15	1.1
	4	0.00	-0.49	-0.25	-0.34	0.02	-0.15	1.45	1.17	-4.8
	5	-0.06	-0.08	-0.12	-0.12	-0.21	-0.22	1.19	1.18	-0.2
	6	0.04	0.21	-0.10	-0.07	0.16	0.23	0.18	0.22	1.7
	7	0.00	0.09	0.10	0.12	0.24	0.29	0.34	0.35	0.9
	8	0.06	-0.02	-0.05	-0.07	0.18	0.13	0.41	0.40	-0.9
	9	0.00	-0.34	0.00	-0.07	0.19	0.01	0.28	0.24	-3.3
	10	-0.17	-0.43	0.14	0.09	0.39	0.27	-	-	-2.4
	11	0.01	0.48	0.00	0.08	0.42	0.61	0.10	0.16	4.3
	12	-0.05	-0.09	0.00	-0.01	0.37	0.35	-0.22	-0.23	-0.4
	13	0.19	0.75	0.00	0.11	0.08	0.26	1.16	1.48	4.7
	14	0.17	0.20	-0.07	-0.07	0.83	0.85	0.50	0.51	0.3
	15	-0.02	0.55	-0.19	-0.07	0.06	0.37	0.50	0.77	5.4
	16	0.13	0.15	0.04	0.04	0.37	0.38	0.79	0.80	0.2
	17	-0.09	-0.64	-0.04	-0.15	0.42	0.19	0.16	-0.09	5.1
F7	1	-0.04	1.66	-0.01	0.29	0.14	1.41	0.52	1.10	15.8
	2	-0.01	1.59	0.05	0.31	-0.50	0.25	0.46	0.70	14.8
	3	0.03	0.74	0.11	0.25	0.31	0.76	-0.24	-0.08	7.1
	4	-0.15	0.56	0.00	0.15	0.17	0.63	0.37	0.65	6.7
	5	0.25	1.09	0.05	0.14	0.00	0.43	0.20	0.53	7.9
	6	-0.28	0.68	0.00	0.21	0.07	0.52	0.51	0.61	10.0
	7/									

Table A50 (Cont'd)

Sheep No.	Sample No.	Plasma Calcium A*	Plasma Calcium C*	Plasma Magnesium A*	Plasma Magnesium C*	Plasma Inorganic P. A*	Plasma Inorganic P. C*	Plasma Citric Acid A*	Plasma Citric Acid C*	Percentage Haemoglobin Difference
	7	0.10	0.30	0.04	0.08	-0.19	-0.11	-0.02	0.00	2.0
	8	0.10	1.68	0.11	0.37	0.06	0.51	0.31	0.47	15.5
	9	0.01	1.18	0.13	0.34	0.04	0.70	0.23	0.33	10.5
	10	-0.39	0.33	-0.01	0.14	0.72	1.13	0.58	0.85	6.8
	11	0.08	2.34	0.05	0.41	-0.08	0.58	0.42	0.68	21.5
	12	-0.13	0.60	-0.02	0.09	-0.17	0.04	0.26	0.29	6.6
	13	0.00	1.44	0.05	0.28	0.42	1.37	0.15	0.24	14.1
	14	-0.20	1.33	-0.01	0.22	0.07	0.90	0.34	0.44	14.5
	15	0.39	1.02	0.02	0.09	-0.09	0.12	0.35	0.39	6.1
	16	0.05	0.46	0.08	0.13	0.20	0.33	0.15	0.16	4.1
F8	1	0.24	1.96	0.00	0.29	-0.18	1.13	0.45	0.96	15.0
	2	0.10	0.18	0.01	0.02	0.05	0.10	-0.11	-0.09	0.7
	3	-0.08	-0.19	0.09	0.07	0.15	0.10	0.17	0.12	-1.0
	4	0.09	1.04	-0.04	0.14	-0.16	0.25	-0.10	0.18	9.5
	5	0.12	0.56	0.04	0.12	0.18	0.44	0.25	0.45	4.1
	6	0.00	-0.21	0.00	0.01	0.32	0.19	0.83	0.73	-2.0
	7	-0.03	0.70	-0.04	0.09	0.65	1.05	0.33	0.65	6.8
	8	-0.03	1.45	0.07	0.35	-0.32	0.30	0.04	0.61	13.5
F9	1	0.08	1.27	0.00	0.29	0.09	0.86	0.40	0.96	11.6
	2	-0.09	1.08	-0.06	0.17	-0.12	0.48	0.12	0.79	11.9

* A - Apparent C - Corrected

Data derived from Table A48.

TABLE A51

Mean Percentage Haemoglobin Differences and Veno-Arterial Differences for Castrated Male Sheep

(all values mg./100 ml.)

Animal Number	M1	M2	M3	M4	M5	M6	M7	Mean
No. of Samples	12	18	1	4	8	6	6	
V-A Differences								
Percentage Haemoglobin	2.0	4.8	3.7	4.6	1.8	4.9	4.6	3.7
Plasma Calcium								
Apparent	-0.06	-0.13	-0.14	0.00	0.01†	0.02	-0.01	-0.055
Corrected	0.14	0.32	0.28	0.49	0.16†	0.48	0.45	0.301
Plasma Magnesium								
Apparent	0.08	0.08	0.04	-0.09	-0.01†	0.03	0.03	0.045
Corrected	0.12	0.19	0.12	0.01	0.02†	0.15	0.14	0.127
Plasma Inorganic Phosphate								
Apparent	0.19	0.23	-0.15	0.07	0.24†	0.39	0.22	0.218
Corrected	0.26	0.57	0.05	0.28	0.35†	0.70	0.53	0.450
Plasma Citric Acid								
Apparent	0.18	0.17†	-0.10	0.16	0.35†	0.09	0.31	0.194
Corrected	0.23	0.27†	0.06	0.24	0.40†	0.17	0.40	0.272

† One value missing from the mean.

Data derived from Table A49.

TABLE A52

Mean Percentage Haemoglobin Differences and Veno-Arterial Differences for Female Sheep

(all values mg./100 ml.)

Animal Number	F1	F2	F3	F4	F5	F6	F7	F8	F9	Mean
Number of Samples	4	6	3	2	4	17	16	8	2	
V-A Differences										
Percentage Haemoglobin	5.2	3.7	5.4	16.7	9.7	-0.1	10.3	5.8	11.8	5.9***
Plasma Calcium										
Apparent	-0.40	-0.08	-0.12	0.10	-0.15†	0.00†	-0.01	0.05	-0.01	-0.042**
Corrected	0.14	0.30	0.38	1.71	1.15†	0.00†	1.06	0.69	1.18	0.587***
Plasma Magnesium										
Apparent	-0.04	-0.01	0.08	0.01	-0.01†	-0.04†	0.03	0.02	-0.03	-0.000
Corrected	0.09	0.09	0.18	0.28	0.23†	-0.04†	0.22	0.14	0.23	0.118***
Plasma Inorganic Phosphate										
Apparent	0.03	0.18	0.27	-0.13	0.00†	0.22†	0.07	0.09	-0.02	0.119
Corrected	0.38	0.36	0.54	0.71	0.79†	0.22†	0.60	0.45	0.67	0.451
Plasma Citric Acid										
Apparent	0.19	0.18	0.74	-0.03	0.46†	0.48††	0.29	0.23	0.26	0.335
Corrected	0.29	0.20	0.78	0.08	0.65†	0.52††	0.46	0.45	0.88	0.465

† and †† One or two values missing from the mean.

, * Individual animal means differ at 1 per cent and 0.1 per cent levels of significance respectively.

Data derived from Table A50.

TABLE A53

Analyses of Variance on Calcium, Magnesium, Inorganic Phosphate and Citric Acid Apparent V-A Differences of Male and Female Sheep

V-A Difference	Source of Variation	Male Sheep				Female Sheep			
		d.f.	Sums of Squares	Mean Square	F	d.f.	Sums of Squares	Mean Square	F
Plasma Calcium	Between Sheep	6	0.2023	0.0337	0.87	8	0.7177	0.0897	2.96**
	Within Sheep	47	1.8170	0.0386		51	1.5456	0.0303	
	Total	53	2.0193			59	2.2633		
Plasma Magnesium	Between Sheep	6	0.1275	0.0212	1.36	8	0.0773	0.0096	1.45
	Within Sheep	47	0.7345	0.0156		51	0.3389	0.0066	
	Total	53	0.8620			59	0.4162		
Plasma Inorganic Phosphate	Between Sheep	6	0.4154	0.0692	1.54	8	0.5336	0.0667	0.89
	Within Sheep	47	2.1161	0.0450		51	3.8301	0.0751	
	Total	53	2.5315			59	4.3637		
Plasma Citric Acid	Between Sheep	6	0.4236	0.0706	0.79	8	1.4414	0.1801	1.72
	Within Sheep	46	4.1955	0.0892		50	5.2299	0.1045	
	Total	52	4.6191			58	6.6713		

Derived from Tables A49 and A50.

TABLE A54

Analyses of Variance on Percentage Haemoglobin Difference, and Calcium, Magnesium, Inorganic Phosphate and Citric Acid Corrected V-A Differences of Male and Female Sheep

V-A Difference	Source of Variation	Male Sheep				Female Sheep			
		d.f.	Sums of Squares	Mean Square	F	d.f.	Sums of Squares	Mean Square	F
Percentage Haemoglobin	Between Sheep	6	105	17.5	0.90	8	1295	161.9	7.60***
	Within Sheep	48	931	19.4		53	1128	21.3	
	Total	54	1036			61	2423		
Plasma Calcium	Between Sheep	6	0.9277	0.1546	0.66	8	14.7623	1.8452	6.92***
	Within Sheep	47	11.0546	0.2352		51	13.5934	0.2665	
	Total	53	11.9823			59	28.3557		
Plasma Magnesium	Between Sheep	6	0.2175	0.0362	1.73	8	0.6980	0.0872	5.59***
	Within Sheep	47	0.9823	0.0209		51	0.8004	0.0156	
	Total	53	1.1998			59	1.4984		
Plasma Inorganic Phosphate	Between Sheep	6	1.4317	0.2386	1.87	8	1.8713	0.2339	1.81
	Within Sheep	47	6.0042	0.1277		51	6.5965	0.1293	
	Total	53	7.4359			59	8.4678		
Plasma Citric Acid	Between Sheep	6	0.4246	0.0708	0.66	8	1.6131	0.2016	1.66
	Within Sheep	46	4.9327	0.1072		50	6.0852	0.1217	
	Total	52	5.3573			58	7.6983		

Derived from Tables A49 and A50.

TABLE A55

Comparison of corrected veno-arterial differences in whole blood obtained by direct estimation and indirectly from plasma analysis.

(*a*) Calcium, magnesium, inorganic phosphate and citric acid values for blood and plasma samples obtained from two sheep by split sample technique

Sheep and sample	Specimen	Calcium*†		Magnesium*†		Inorganic phosphate*		Citric acid*	
		A	V	A	V	A	V	A	V
B 1	Blood	7·33	7·26	3·03	3·01	7·10	7·18	1·77	1·95
	Plasma	9·62	9·40	2·61	2·57	8·06	8·28	2·10	2·30
2	Blood	6·91	6·84	3·04	3·01	7·64	7·62	1·74	1·97
	Plasma	8·95	9·05	2·67	2·61	8·64	9·00	2·41	2·62
3	Blood	5·81	5·97	3·25	3·16	5·55	5·55	0·50	0·62
	Plasma	8·22	8·17	2·48	2·41	6·51	6·64	0·58	0·67
4	Blood	5·78	5·95	3·16	3·16	5·34	5·40	0·53	0·60
	Plasma	8·24	8·46	2·45	2·45	6·24	6·24	0·50	0·60
C 1	Blood	..	..	..	..	5·55	6·37	..	..
	Plasma	..	..	..	..	6·27	7·65	..	..
2	Blood	..	..	..	..	5·71	6·37	..	..
	Plasma	..	..	..	..	6·80	7·51	..	..
3	Blood	..	..	..	..	5·76	6·08	..	..
	Plasma	..	..	..	..	6·86	7·20	..	..
4	Blood	..	..	..	..	5·41	5·53	..	..
	Plasma	..	..	..	..	6·61	6·73	..	..
5	Blood	..	..	..	..	5·37	5·80	..	..
	Plasma	..	..	..	..	6·29	6·88	..	..
6	Blood	..	..	..	..	5·23	5·71	..	..
	Plasma	..	..	..	..	6·27	6·71	..	..
7	Blood	..	..	..	..	4·96	5·52	1·42	1·40
	Plasma	..	..	..	..	6·27	6·73	1·40	1·36
8	Blood	..	..	..	..	4·88	5·23	1·38	1·46
	Plasma	..	..	..	..	5·60	6·31	1·54	1·69
9	Blood	7·97	7·88	2·61	2·63	4·82	5·40	2·30	2·66
	Plasma	10·74	10·70	2·03	1·98	5·54	6·40	2·94	3·19
10	Blood	7·55	7·61	2·58	2·59	4·68	5·30	1·62	1·57
	Plasma	10·01	10·16	2·17	2·08	5·08	6·03	1·87	1·90

* mg./100 ml. blood or plasma. All estimations in triplicate.
† Whole blood estimations carried out on trichloroacetic acid filtrates by the method already described, after neutralization to phenolphthalein.

(*b*) Hæmoglobin ratios and changes in cell volume of blood samples from two sheep

Sheep and sample	Hæmoglobin reading		Hæmoglobin ratio	Total cell volume*		Hæmoglobin reading per unit of cell volume		Relative change in cell volume†
	A	V	A/V	A	V	A	V	
B 1	1·113	1·111	1·002	24·4	26·6	0·0456	0·0418	1·092
2	1·145	1·129	1·014	25·2	24·6	0·0454	0·0459	0·990
3	1·036	1·056	0·981	20·8	23·4	0·0498	0·0451	1·102
4	1·071	1·109	0·966	23·4	24·5	0·0458	0·0453	1·013
C 1	1·459	1·490	0·979	..	..	..	..	..
2	1·372	1·324	1·036	..	..	..	..	..
3	1·298	1·288	1·008	..	..	..	..	..
4	1·375	1·361	1·010	..	..	..	..	..
5	1·328	1·333	0·996	..	..	..	..	..
6	1·252	1·245	1·006	..	..	..	..	..
7	1·207	1·200	1·006	23·7	25·9	0·0455	0·0429	1·060
8	1·120	1·139	0·983	24·8	26·0	0·0452	0·0428	1·056
9	1·127	1·163	0·970	22·5	25·1	0·0501	0·0463	1·081
10	1·132	1·126	1·005	22·7	23·8	0·0498	0·0473	1·052
Mean of B 1–4 and C 7–10	..	..	0·991	..	..	..	..	1·056

* Evans blue method of Fisher [1962].
† Arterial hæmoglobin reading per unit of cell volume/Venous hæmoglobin reading per unit of cell volume.

The results indicate that the cells increase in size on passing from the artery to the portal vein and that this increase is independent of change in the hæmoglobin ratio.

(*c*) Corrected veno-arterial differences for whole blood obtained by direct estimation and indirectly* by plasma analysis

Sheep and sample	Veno-Arterial differences							
	Calcium†		Magnesium†		Inorganic phosphate†		Citric acid†	
	Direct	Indirect	Direct	Indirect	Direct	Indirect	Direct	Indirect
B 1	−0·06	−0·15	−0·02	−0·02	0·09	0·18	0·18	0·15
2	0·02	0·15	0·01	−0·02	0·09	0·37	0·26	0·18
3	0·05	−0·17	−0·15	−0·10	−0·11	0·00	0·11	0·06
4	−0·03	−0·05	−0·11	−0·06	−0·12	−0·16	0·05	0·06
C 1	..	..	..	..	0·70	0·85	..	..
2	..	..	..	..	0·88	0·69	..	..
3	..	..	..	..	0·36	0·28	..	..
4	..	..	..	..	0·18	0·14	..	..
5	..	..	..	..	0·41	0·40	..	..
6	..	..	..	..	0·51	0·35	..	..
7	..	..	..	..	0·59	0·38	−0·01	−0·03
8	..	..	..	..	0·26	0·45	0·06	0·09
9	−0·33	−0·28	−0·06	−0·09	0·42	0·52	0·28	0·12
10	0·10	0·15	0·02	−0·06	0·65	0·76	−0·04	0·03
Mean	−0·04	−0·06	−0·05	−0·06	0·35	0·37	0·11	0·08

* The indirect method entailed estimating the corrected Veno-Arterial difference per 100 ml. plasma and correcting for cell volume.
† mg./100 ml. blood.
The differences between the direct and indirect methods were smaller than the expected analytical error.

TABLE A56

Plasmas and Lambs Used for Queensland Transfusion Experiments

(Normal Calving Cows)

Group	Donor Cow No.	Plasma Transfused				Wt. of Lamb (lb.)	Volume Plasma Injected (ml.)
		Time of Collection before (-) or after (+) calving	No. of Calving	Composition Calcium mg/100 ml.	Inorganic P. mg/100 ml.		
1. Control	Q1	- 5d		10.40	6.03	29	70
	Q2	- 5d	3	12.50	6.12	31	75
	Q3	- 7d		10.83	3.36	34	80
	Q4	- 5d	4	10.00	4.37	36	75
	Q5	-10d	6	10.23	4.20	30	75
	Q6	- 2d	2	10.90	6.35	39	90
	Q7	-10d	4	10.94	6.28	30	75
	Q8	- 5d	4	10.50	6.24	42	105
2. Parturient	Q1	+30min			3.40	32	70
	Q2	- 1hr	3	11.02	4.00	28	70
	Q3	+10min		10.50	4.03	36	80
	Q4	+ 7min	4	9.13	6.00	36	75
	Q5	+10min	6	10.38	5.70	30	75
	Q7	+15min	4	7.92	3.54	30	75
	Q8	+15min	4	12.20	5.94	31	77
3. Post-Partum	Q2	+12hr	3	9.75	5.38	34	75

TABLE A57

Plasmas and Lambs Used for Scottish Transfusion Experiments

Group	Donor Cow No.	Plasma Transfused: Time of Collection before (-) or after (+) calving	Plasma Transfused: No. of Calving	Plasma Transfused: Composition Calcium mg/100 ml.	Plasma Transfused: Composition Inorganic P. mg/100 ml.	Wt. of Lamb (lb.)	Volume Plasma Injected (ml.)
1.							
Control	S1	+14d	5	9.45	5.93	63	126
	S2	+ 4d	3	9.05	4.43	37	75
	S3	- 3mth	4	10.30	5.75	81	240
	S4	+ 6d	6	9.05	6.04	58	116
	S5	+ 6d	4	10.90	4.07	45	90
2.							
Parturient	S1*	- 1hr	5	5.65	2.50	60	120
	S2	+ 1hr	3	8.50	3.01	44	88
	S4	+2½hr	6	9.15	2.50	53	106
	S5	- 1hr	4	8.70	1.78	52	104
	S6	0hr	3	5.13	1.69	3ml/lb	live wt.
	S7	+ ½hr	3	8.70	5.80	70	150
3.							
One day Post-Partum	S1*	+24hr	5	4.30	3.65	60	120
	S2	+33hr	3	10.40	5.83	35	70
	S4	+26hr	6	8.25	4.75	56	112
	S6	+24hr	3	5.45	3.42	3ml/lb	live wt.
	S7	+24hr	3	9.05	5.75	84	170
Repeat Transfusions, Sampling Before and at 24 hours only							
Control	S2	+ 4d	3	9.05	4.43	36	72
	S4	+ 6d	6	9.05	6.04	58	116
Parturient	S2	+ 1hr	3	8.50	3.01	52	105
	S4	+2½hr	6	9.15	2.50	53	106
Post-Partum	S2	+33hr	3	10.40	5.83	41	82
Other Transfusions, Sampling Before and at 24 hours only							
Control	S8	+12d	3	9.20	2.56	55	110
	S8	+13d	3	11.40	3.66	55	110
	S9	+ 1mth	6		4.47	50	100
	S10	+ 1mth	4		5.92	29	60
	S11	Preg.Non-	1		6.75	30	65
	S12	lactating	1		4.20	32	70
	S13	Do.	4		6.00	40	85

* Milk Fever

TABLE A58

Plasma Calcium and Inorganic Phosphate Values of Lambs Transfused with Bovine Plasma (Scottish Repeat and Other Experiments, Sampling Before and at Twenty-four Hours Only).

mg/100 ml.

Group	Donor Cow No.	Plasma Calcium		Plasma Inorganic Phosphate	
		Before	24 Hrs.After	Before	24 Hrs.After
Control	S2	9.90	10.90	5.00	4.23
	S4	10.85	10.70	4.43	3.93
Parturient	S2	9.65	8.80	4.15	3.58
	S4	10.05	10.05	3.21	3.42
Post-partum	S2	9.75	9.30	3.86	4.02
Control	S8	10.55	10.45	3.00	3.13
	S8	10.95	10.45	3.32	3.62
	S9			5.25	5.45
	S10			3.90	3.52
	S11			3.45	3.78
	S12			4.00	4.60
	S13			3.35	3.30

TABLE A59

Serum Calcium Values for Queensland Cows at Normal Calving

mg/100 ml.

Cow No.	No.of Calving	Days from Calving							
		2	1	0	½	1	1½	2	3
Q4	1	10.00	9.38	9.13	9.90	10.00	9.93	8.50	8.69
Q5	6	10.80	(11.69)	10.38	10.66	10.33	9.81	10.33	10.23
Q7	4	10.33	9.62	7.94	6.85	6.38	6.85	8.22	9.86
Q8	4	9.03	12.69	12.20	10.00	10.00	10.49	9.64	10.00

Plasma Inorganic Phosphate Values for Queensland Cows at Normal Calving

mg/100 ml.

Cow No.	No.of Calving	Days from Calving							
		2	1	0	½	1	1½	2	3
Q4	1	4.85	5.13	6.00	6.98	6.30	6.20	6.88	7.23
Q5	6	6.00	(7.61)	5.70	7.45	9.63	8.55	8.53	10.63
Q7	4	4.55	6.56	3.54	5.51	5.97	6.23	7.40	5.40
Q8	4	5.63	6.41	5.94	7.54	8.50	8.00	9.54	8.13

TABLE A60

Plasma Calcium Values of Lambs Transfused with Bovine Plasma (Queensland Experiments)

mg/100 ml.

Group	Donor Cow No.	Time after Transfusion: Pre	10mins	1 hr	4 hr	12 hr	24 hr	36 hr	48 hr	Mean
1. Control	Q1	12.03	12.72	11.61	10.71		11.55			
	Q2	12.08	12.19	12.24	12.37		12.72			
	Q3	-	-	-	-		-			
	Q4	11.77	(10.47)	10.50	11.30	10.94	10.71	10.95	10.62	10.91
	Q5	10.42	7.98	8.92	9.15	10.28	8.69	10.61	10.05	9.51
	Q6	9.90	9.25	9.60	10.10	10.00	8.60	10.70	9.30	9.68
	Q7	10.00	8.92	(8.56)	8.31	9.35	8.45	8.17	9.11	8.86
	Q8	9.64	10.19	9.94	9.88	10.25	9.64	10.25	10.61	10.05
Q4 - Q8	Mean	10.35	9.36	9.50	9.75	10.16	9.22	10.14	9.94	9.80
2. Parturient	Q1	11.87	12.61	9.89	12.30					
	Q2	12.14	12.30	11.50	11.13	(11.90)	11.87	12.72	12.51	12.01
	Q3	13.60	13.90	12.50	13.35	13.50	13.60	14.25	13.95	13.58
	Q4	12.48	11.45	10.95	11.29	11.38	11.60	11.19	11.05	11.42
	Q5	12.90	12.75	12.50	12.10	11.90	11.75	12.10	11.35	12.17
	Q7	10.95	10.52	10.47	10.34	10.56	10.04	10.78	9.87	10.44
	Q8	10.00	10.00	9.57	9.33	9.63	9.76	11.70	9.35	9.92
Q2 - Q8	Mean	12.01	11.82	11.25	11.26	11.48	11.44	12.12	11.35	11.59
1 and 2	Mean	11.25	10.70	10.46	10.57	10.88	10.43	11.22	10.71	10.78
3. Post-Partum	Q2	12.35	12.40	12.03	11.93			12.19	11.98	

Analysis of Variance
Groups 1 and 2

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	1	69.7677	69.7677	7.21*
Within Groups	9	87.1332	9.6814	
Animals	10	156.9009		
Times	7	7.8445	1.1206	4.35***
Groups x Times	7	3.1017	0.4431	1.72
Remainder	60	15.4602	0.2576	
Total	84	183.3073		

Values in brackets are calculated.

TABLE A61

Plasma Calcium Values of Lambs Transfused with Bovine Plasma (Scottish Experiments)

mg/100 ml.

Group	Donor Cow No.	Time after Transfusion: Pre	10mins	1 hr	2 hr	4 hr	6 hr	12 hr	18 hr	24 hr	36 hr	48 hr	Mean
1. Control	S1	11.15	11.15	11.65	10.15	9.65	10.50	8.55	8.35	8.70	9.40	9.50	9.89
	S2	7.90	7.45	8.20	8.30	8.80	8.20	8.25	(7.71)	8.25	8.50	7.50	8.12
	S3	11.80	11.30	11.90	11.25	10.90	11.20	10.15	9.70	9.90	10.50	10.30	10.81
	S4	8.00	8.10	8.10	8.10	8.25	8.90	9.10	9.30	9.40	9.45	9.70	8.76
	S5	11.60	11.30	11.20	11.60	11.10	11.10	11.40	11.70	(10.81)	10.60	10.10	11.14
	Mean	10.09	9.86	10.21	9.88	9.74	9.98	9.49	9.35	9.41	9.69	9.42	9.74
2. Parturient	S1	9.80	9.85	9.95	10.25	10.30	9.50	9.90	10.17	10.30	11.10	10.70	10.17
	S2	10.50	10.85	10.55	10.80	10.00	10.25	10.20	10.30	10.40	10.10	9.75	10.34
	S4	11.15	11.10	10.90	10.85	10.35	10.50	9.55	9.90	9.65	9.90	9.60	10.31
	S5	10.70	10.10	11.80	11.10	11.50	11.10	10.80	(11.07)	10.85	10.65	11.00	10.97
	S6	7.50	7.20	7.15	7.30	7.60	7.50	7.60	7.85	8.05	8.40	8.95	7.74
	S7	10.60	10.40	10.40	10.70	10.60	10.90	11.20	11.80	11.70	11.50	11.00	10.98
	Mean	10.04	9.92	10.13	10.17	10.06	9.96	9.88	10.18	10.16	10.28	10.18	10.08
3. One Day Post-Partum	S1	9.45	9.35	9.15	8.73	7.83	8.15	7.70	8.40	8.50	9.55	10.07	8.81
	S2	9.85	9.60	9.90	9.95	10.50	10.95	11.20	(10.42)	10.25	10.15	10.35	10.28
	S4	9.40	9.35	9.40	9.40	9.30	9.75	10.30	9.90	9.95	10.75	10.05	9.78
	S6	10.40	10.25	10.70	10.60	10.10	10.75	10.95	11.25	10.40	9.85	10.05	10.57
	S7	11.60	10.65	11.05	11.10	10.50	11.00	10.95	11.20	11.60	11.45	11.45	11.14
	Mean	10.14	9.84	10.04	9.96	9.65	10.12	10.22	10.23	10.14	10.35	10.39	10.10
1,2 & 3	Mean	10.09	9.78	10.13	10.01	9.83	10.02	9.86	9.94	9.92	10.12	10.00	9.98

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	2	4.6876	2.3438	0.16
Within Groups	13	187.3891	14.4145	
Animals	15	192.0767		
Times	10	1.7043	0.1704	0.46
Groups x Times	20	5.9538	0.2976	0.80
Remainder	126	46.9726	0.3727	
Total	171	246.7074		

Values in brackets are calculated.

TABLE A62

Plasma Inorganic Phosphate Values of Lambs Transfused with Bovine Plasma (Queensland Experiments)

mg/100 ml.

Group	Donor Cow No.	Time after Transfusion											
		Pre	10mins	30mins	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	36 hr	48 hr	Mean
1. Control	Q1	7.35	8.17	8.31	7.77	8.06	7.58	5.75		7.75			
	Q2	4.82	4.10	4.52	4.05	2.52	3.30	3.65		5.08			
	Q3	8.11	7.80	(7.10)	7.63	6.50	6.52	6.32	5.63	6.82	7.23	7.43	7.01
	Q4	8.37	8.00	8.00	7.20	6.78	8.00	8.88	7.88	7.75	7.88	9.00	7.98
	Q5	5.91	5.88	5.30	4.98	5.23	5.93	5.60	7.75	6.98	6.38	6.68	6.06
	Q6	9.21	9.68	9.38	8.40	7.20	7.28	6.70	8.85	10.80	9.18	10.35	8.82
	Q7	10.35	9.33	9.68	9.43	9.48	9.88	8.70	9.95	11.05	8.53	9.18	9.60
	Q8	4.36	4.61	4.26	3.77	3.77	3.30	3.12	3.67	3.46	3.28	3.12	3.70
Q3-Q8	Mean	7.72	7.55	7.29	6.90	6.49	6.82	6.55	7.29	7.81	7.08	7.63	7.19
2. Parturient	Q1	4.32	5.15	5.50	4.35	4.25	3.50	3.90	2.84	2.33			
	Q2	5.16	5.67	5.34	4.79	4.42	4.13	3.37	3.85	3.32	4.03	4.68	4.43
	Q3	8.33	9.17	7.55	(6.78)	7.75	7.23	7.72	5.90	6.27	6.90	7.43	7.37
	Q4	6.25	5.90	5.80	6.15	6.05	6.75	5.58	5.65	5.45	5.89	6.40	6.00
	Q5	9.06	8.65	8.98	8.43	8.33	7.90	6.14	8.53	9.00	10.80	11.58	8.85
	Q7	5.09	5.58	5.13	4.35	4.35	4.28	5.83	5.10	4.40	5.95	4.45	4.95
	Q8	2.82	2.70	2.90	2.98	2.86	3.43	2.53	3.25	3.48	2.70	3.20	2.99
Q2-Q8	Mean	6.12	6.28	5.95	5.58	5.63	5.62	5.20	5.38	5.32	6.05	6.29	5.76
1 & 2	Mean	6.92	6.91	6.62	6.24	6.06	6.22	5.87	6.33	6.57	6.56	6.96	6.48
3. Post-Partum	Q2	2.45	2.48	2.23	1.87	2.35	2.07	2.13		3.68	3.27	4.50	

Analysis of Variance

(Groups 1 and 2)

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	1	67.4283	67.4283	1.37
Within Groups	10	493.5115	49.3512	
Animals	11	560.9398		
Times	10	15.9903	1.5990	2.59**
Groups x Times	10	5.8993	0.5899	0.95
Remainder	98	60.5954	0.6183	
Total	129	643.4248		

Values in brackets are calculated.

TABLE A63

Plasma Inorganic Phosphate Values of Lambs Transfused with Bovine Plasma (Scottish Experiments)

mg/100 ml.

Group	Donor Cow No.	Time after Transfusion: Pre	10mins	1 hr	2 hr	4 hr	6 hr	12 hr	18 hr	24 hr	36 hr	48 hr	Mean
1. Control	S1	5.77	5.90	5.50	4.77	4.10	4.55	5.38	5.38	5.38	5.10	4.77	5.15
	S2	8.50	7.20	6.00	5.85	6.35	5.58	7.45	(7.71)	8.35	8.73	10.95	7.52
	S3	5.00	4.55	4.15	4.13	4.35	3.35	5.08	3.55	3.60	3.83	3.95	4.14
	S4	6.28	6.19	5.50	6.30	6.17	6.02	6.09	5.34	4.94	4.36	5.47	5.70
	S5	4.72	4.16	3.72	3.89	4.87	4.56	5.08	6.08	5.08	3.89	4.33	5.58
	Mean	6.05	5.60	4.97	4.99	5.17	4.81	5.82	5.61	5.47	5.18	5.89	5.42
2. Parturient	S1	5.52	5.40	4.95	5.45	5.38	5.85	5.07	4.65	4.05	3.80	4.20	4.94
	S2	6.93	7.87	6.20	5.50	5.08	6.30	6.68	6.48	6.35	7.00	6.72	6.46
	S4	7.90	7.90	6.77	6.98	5.95	5.00	6.60	(5.88)	(5.71)	6.13	5.63	6.40
	S5	9.20	8.70	6.85	6.75	7.72	7.50	7.13	(6.88)	6.70	6.55	7.50	7.41
	S6	5.43	5.78	4.99	4.17	3.85	3.95	4.66	4.09	3.88	4.29	4.42	4.50
	S7	5.65	5.96	5.48	5.25	4.38	4.38	4.88	3.53	3.79	5.73	5.42	4.95
	Mean	6.77	6.94	5.87	5.68	5.39	5.50	5.84	5.25	5.08	5.58	5.65	5.78
3. One Day Post-Partum	S1	8.33	8.55	7.65	6.85	6.80	7.40	8.30	7.38	6.03	5.53	5.40	7.11
	S2	8.20	6.98	5.25	5.40	6.18	6.18	6.90	(6.22)	4.85	5.18	4.43	5.98
	S4	5.05	5.10	4.35	4.40	4.55	4.75	4.58	4.07	4.06	3.46	4.11	4.41
	S6	7.20	6.30	5.70	5.30	4.88	5.88	6.43	7.35	8.00	6.30	6.70	6.37
	S7	6.90	7.75	6.30	7.13	6.48	5.77	6.37	7.00	8.18	8.00	6.60	6.95
	Mean	7.14	6.94	5.85	5.82	5.78	6.00	6.52	6.40	6.22	5.69	5.45	6.16
1,2 & 3	Mean	6.66	6.52	5.59	5.51	5.44	5.44	6.04	5.72	5.56	5.49	5.66	5.79

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	2	15.3920	7.6960	0.50
Within Groups	13	199.1791	15.3214	
Animals	15	214.5711		
Times	10	30.0881	3.0088	12.84***
Groups x Times	20	58.6870	2.9343	12.52***
Remainder	125	29.2876	0.2343	
Total	170	332.6338		

Values in brackets are calculated.

TABLE A64

Changes in Plasma Inorganic Phosphate Values of Lambs Transfused with Bovine Plasma (Queensland Experiments)

mg./100 ml.

Group	Donor Cow No.	Time after Transfusion 10mins	30mins	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	36 hr	48 hr	Mean
1. Control	Q1	+0.82	+0.94	+0.42	+0.71	+0.23	-1.60		+0.40			
	Q2	-0.72	-0.30	-0.77	-2.30	-1.52	-1.17		+0.26			
	Q3	-0.31	(-1.01)	-0.48	-1.61	-1.59	-1.79	-2.48	-1.29	-0.88	-0.68	-1.21
	Q4	-0.37	-0.37	-1.17	-1.59	-0.37	+0.51	-0.49	-0.62	-0.49	+0.63	-0.43
	Q5	-0.03	-0.61	-0.93	-0.68	+0.02	-0.31	+1.84	+1.07	+0.47	+0.77	0.16
	Q6	+0.47	+0.17	-0.81	-2.01	-1.93	-2.51	-0.36	+1.59	-0.03	+1.14	-0.43
	Q7	-1.02	-0.65	-0.92	-0.87	-0.47	-1.65	-0.40	+0.70	-1.82	-1.17	-0.83
	Q8	+0.25	-0.10	-0.59	-0.59	-1.06	-1.24	-0.69	-0.90	-1.08	-1.24	-0.72
Q3-Q8	Mean	-0.17	-0.43	-0.82	-1.23	-0.90	-1.17	-0.43	+0.09	-0.64	-0.09	-0.58
2. Partur-ient	Q1	+0.83	+1.18	+0.03	-0.07	-0.82	-0.42	-1.48	-1.99			
	Q2	+0.51	+0.18	-0.37	-0.74	-1.03	-1.79	-1.31	-1.84	-1.13	-0.48	-0.80
	Q3	+0.84	-0.78	(-1.55)	-0.58	-1.08	-0.61	-2.43	-2.06	-1.43	-0.90	-1.06
	Q4	-0.35	-0.45	-0.10	-0.20	+0.50	-0.67	-0.60	-0.80	-0.36	+0.15	-0.29
	Q5	-0.41	-0.08	-0.63	-0.73	-1.16	-2.92	-0.53	-0.06	+1.74	+2.52	-0.23
	Q7	+0.49	+0.04	-0.74	-0.74	-0.81	+0.74	+0.01	-0.69	+0.86	-0.64	-0.15
	Q8	-0.12	+0.08	+0.16	+0.04	+0.61	-0.29	+0.43	+0.66	-0.12	+0.38	0.18
Q2-Q8	Mean	+0.16	-0.17	-0.54	-0.49	-0.50	-0.92	-0.74	-0.80	-0.07	+0.17	-0.39
1 and 2	Mean	0.00	-0.30	-0.68	-0.86	-0.70	-1.04	-0.57	-0.35	-0.36	+0.04	-0.48
3. Post-partum	Q2	+0.03	-0.22	-0.58	-0.10	-0.38	-0.32		+1.23	+0.82	+2.05	

Analysis of Variance (Groups 1 and 2)

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	1	1.0565	1.0565	0.50
Within Groups	10	21.1344	2.1134	
Animals	11	22.1909		
Times	9	13.4370	1.4930	2.24*
Groups x Times	9	5.8100	0.6455	
Remainder	88	58.6716	0.6667	0.97
Total	117	100.1095		

Values in brackets are calculated.

Data derived from Table A62.

TABLE A65

Changes in Plasma Inorganic Phosphate Values of Lambs Transfused with Bovine Plasma (Scottish Experiments)

mg/100 ml.

Group	Donor Cow No.	Time after Transfusion 10mins	1 hr	2 hr	4 hr	6 hr	12 hr	18 hr	24 hr	36 hr	48 hr	Mean
1. Control	S1	+0.13	-0.27	-1.00	-1.67	-1.22	-0.39	-0.39	-0.39	-0.67	-1.00	-0.69
	S2	-1.30	-2.50	-2.65	-2.15	-2.92	-1.05	(-0.79)	-0.15	+0.23	+2.45	-1.08
	S3	-0.45	-0.85	-0.87	-0.65	-1.65	+0.08	-1.45	-1.40	-1.17	-1.05	-0.95
	S4	-0.09	-0.78	+0.02	-0.11	-0.26	-0.19	-0.94	-1.36	-1.92	-0.81	-0.64
	S5	-0.56	-1.00	-0.83	+0.15	-0.16	+0.36	+1.36	+0.36	-0.83	-0.39	-0.15
	Mean	-0.45	-1.08	-1.07	-0.89	-1.24	-0.24	-0.44	-0.59	-0.87	-0.16	-0.70
2. Partur-ient	S1	-0.12	-0.57	-0.07	-0.14	+0.33	-0.45	-0.87	-1.47	-1.72	-1.32	-0.64
	S2	+0.94	-0.73	-1.43	-1.85	-0.63	-0.25	-0.45	-0.58	+0.07	-0.21	-0.51
	S4	0.00	-1.13	-0.92	-1.95	-2.90	-1.30	(-2.02)	(-2.19)	-1.77	-2.27	-1.64
	S5	-0.50	-2.35	-2.45	-1.48	-1.70	-2.07	(-2.32)	-2.50	-2.65	-1.70	-1.97
	S6	+0.35	-0.44	-1.26	-1.58	-1.48	-0.77	-1.34	-1.55	-1.14	-1.01	-1.02
	S7	+0.31	-0.17	-0.40	-1.27	-1.27	-0.77	-2.12	-1.86	+0.08	-0.23	-0.77
	Mean	+0.16	-0.90	-1.09	-1.38	-1.28	-0.94	-1.52	-1.69	-1.19	-1.12	-1.09
3. One day Post-Partum	S1	+0.22	-0.68	-1.48	-1.53	-0.93	-0.03	-0.95	-2.30	-2.80	-2.93	-1.34
	S2	-1.22	-2.95	-2.80	-2.02	-2.02	-1.30	(-1.98)	-3.35	-3.02	-3.77	-2.44
	S4	+0.05	-0.70	-0.65	-0.50	-0.30	-0.47	-0.98	-0.99	-1.59	-0.94	-0.71
	S6	-0.90	-1.50	-1.90	-2.32	-1.32	-0.77	+0.15	+0.80	-0.90	-0.50	-0.92
	S7	+0.85	-0.60	+0.23	-0.42	-1.13	-0.53	+0.10	+1.28	+1.10	-0.30	+0.06
	Mean	-0.20	-1.29	-1.32	-1.36	-1.14	-0.62	-0.73	-0.91	-1.44	-1.69	-1.07
1,2 & 3	Mean	-0.14	-1.08	-1.15	-1.22	-1.22	-0.62	-0.94	-1.10	-1.17	-1.00	-0.96

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	2	4.9771	2.4886	0.58
Within Groups	13	56.2452	4.3265	
Animals	15	61.2223		
Times	9	16.5802	1.8422	4.29***
Group x Time	18	34.3004	1.9055	4.43***
Remainder	112	48.1300	0.4297	
Total	154	160.2329		

Values in brackets are estimated.

Data derived from Table A63.

TABLE A66

Plasmas and Lambs Used for Queensland Transfusion Experiments

(Milk Fever Cows)

Group	Donor Cow No.	Plasma Transfused				Wt. of Lamb (lb.)	Volume Plasma Injected (ml.)
		Time of Collection after Calving	No. of Calving	Composition Calcium mg/100 ml.	Composition Inorganic P. mg/100 ml.		
1. Before Treatment	MF1	3d 12hrs	4	3.33	2.20	27	65
	MF2		5	3.14	1.05	36	75
	MF3			5.20	1.45		75
2. After Recovery	MF1	4d	4	6.69	6.08	29	65
	MF2	4½d	5	10.36	4.25	30	75
	MF3			9.84	4.46		75

TABLE A67

Plasma Calcium Values of Lambs Transfused with Plasma from Cows Before and After Milk Fever (Queensland)

mg/100 ml.

Group	Donor Cow No.	Time after Transfusion: Pre	5mins	1 hr	4 hr	12 hr	24 hr	36 hr	48 hr	Mean
1. Before Treatment	MF1	10.43	10.76	10.00	10.38	9.90	9.74	10.52	10.33	10.26
	MF2	8.77	8.95	8.55	9.18	9.23	9.05	10.91	11.23	9.48
	MF3	11.33	(11.56)	11.72	10.94	12.07	11.95	11.72	11.33	11.58
	Mean	10.18	10.42	10.09	10.17	10.40	10.25	11.05	10.96	10.44
2. After Treatment	MF1	10.36	10.24	9.52	9.48	9.90	10.33	(10.26)	10.00	10.01
	MF2	8.01	7.95	7.37	7.64	8.68	8.70	9.50	8.41	8.28
	MF3	10.43	(10.62)	9.84	10.70	11.72	11.17	9.96	10.94	10.67
	Mean	9.60	9.60	8.91	9.27	10.10	10.07	9.91	9.78	9.66
1 and 2	Mean	9.89	10.01	9.50	9.62	10.25	10.16	10.48	10.37	10.05

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	1	7.3790	7.3790	0.69
Within Groups	4	42.5142	10.6285	
Animals	5	49.8932		
Times	7	4.6693	0.6670	1.82
Groups x Times	7	1.6471	0.2353	0.64
Remainder	25	9.1633	0.3665	
Total	44	65.3729		

Values in brackets are calculated.

TABLE A68

Plasma Inorganic Phosphate Values of Lambs Transfused with Plasma from Cows Before and After Milk Fever (Queensland)

mg/100 ml.

Group	Donor Cow No.	Time from Transfusion: Pre	5mins	30mins	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	36 hr	48 hr	Mean
1. Before Treatment	MF1	4.94	5.03	4.58	4.45	4.08	3.63	3.93	3.70	4.18	3.68	4.20	4.22
	MF2	10.50	11.30	10.05	8.70	9.05	9.68	9.95	9.15	7.15	6.88	7.06	9.04
	MF3	6.93	7.60	7.10	6.37	5.88	6.22	5.86	6.36	6.03	6.16	6.03	6.41
	Mean	7.46	7.98	7.25	6.51	6.34	6.51	6.58	6.40	5.89	5.57	5.76	6.56
2. After Recovery	MF1	10.84	10.28	9.80	10.10	10.18	9.58	9.80	8.70	9.90	10.23	8.58	9.82
	MF2	13.56	13.48	12.40	12.25	11.25	11.82	11.98	12.08	10.23	7.56	9.24	11.44
	MF3	9.09	9.64	9.50	9.13	7.79	7.44	7.14	7.08	8.27	7.97	8.59	8.33
	Mean	11.16	11.13	10.57	10.49	9.74	9.61	9.64	9.29	9.47	8.59	8.80	9.86
1 & 2	Mean	9.31	9.56	8.91	8.50	8.04	8.06	8.11	7.85	7.63	7.08	6.29	8.21

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	1	180.25	180.25	3.97
Within Groups	4	181.60	45.40	
Animals	5	361.85		
Times	10	38.02	3.80	4.55***
Group x Time	10	1.36	0.14	0.16
Remainder	40	33.43	0.84	
Total	65	434.66		

TABLE A69

Plasma Calcium Values of Lambs Infused with Standard Phosphate Solutions

mg/100 ml.

Group	Inorganic P. in Infusion Solution	Volume Infused ml.	Wt. of Lamb lb.	Time after Infusion: Pre	5 min	1 hr	4 hr	12 hr	24 hr	36 hr	48 hr	Mean
1.	3 mg.%	95	38	9.62	9.80	9.70	9.05	8.80	8.75	9.80	8.63	9.27
		95	36	9.70	9.45	9.34	9.70	9.45	8.85	9.54	8.46	9.31
		92	37	10.50	10.50	10.50	9.75	10.50	10.60	9.70	10.15	10.28
	Mean			9.94	9.92	9.85	9.50	9.58	9.40	9.68	9.08	9.62
2.	7 mg.%	80	32	9.50	10.01	9.60	9.05	9.65	8.35	9.25	8.95	9.29
		85	34	9.70	10.00	10.00	8.92	8.92	9.23	9.70	9.23	9.48
		100	41	10.50	10.20	10.20	10.70	9.00	11.25	10.55	10.80	10.40
	Mean			9.90	10.07	9.93	9.56	9.19	9.61	9.83	9.66	9.72
1&2	Mean			9.92	9.99	9.89	9.53	9.39	9.51	9.76	9.37	9.67

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	1	0.1220	0.1220	0.04
Within Groups	4	10.8564	2.7141	
Animals	5	10.9784		
Times	7	2.6431	0.3775	1.52
Groups x Times	7	0.7699	0.1100	0.44
Remainder	28	6.9456	0.2480	
Total	47	21.3370		

TABLE A70

Plasma inorganic phosphate values of lambs infused with standard phosphate solutions.

mg./100 ml.

Group	Inorganic P. in Infusion Solution	Time after Infusion											
		Pre	5 min.	30 min.	1 hr.	2 hr.	4 hr.	6 hr.	12 hr.	24 hr.	36 hr.	48 hr.	Mean
1	3 mg.%	4.50	4.34	3.58	3.61	3.59	4.23	3.85	6.18	6.43	5.37	4.87	4.60
		5.03	5.02	4.54	5.06	4.65	5.08	5.29	5.29	4.90	5.21	6.01	5.10
		6.23	6.70	6.30	6.56	7.37	3.82	4.30	4.02	4.98	3.16	3.73	5.20
	Mean	5.25	5.35	4.81	5.07	5.20	4.38	4.48	5.16	5.44	4.55	4.87	4.96
2	7 mg.%	4.53	4.46	4.21	4.34	3.68	4.65	5.57	5.27	5.08	5.05	5.52	4.76
		3.73	2.31	2.39	2.42	3.50	3.02	3.29	3.69	3.73	3.23	4.57	3.26
		3.54	3.75	3.69	3.85	3.31	2.56	2.31	2.48	2.37	2.20	3.16	3.02
	Mean	3.93	3.51	3.43	3.54	3.50	3.41	3.72	3.81	3.73	3.49	4.42	3.68
1 and 2	Mean	4.59	4.43	4.12	4.31	4.35	3.89	4.10	4.49	4.58	4.04	4.64	4.32

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	1	27.1618	27.1618	4.98
Within groups	4	21.8363	5.4590	
Animals	5	48.9981		
Times	10	3.8392	0.3839	0.3918
Groups x Times	10	2.7962	0.2796	0.2854
Remainder	40	39.1843	0.9796	
Total	65	94.8178		

TABLE A71

Plasmas and Lambs Used for Ethanol Extraction Experiments

Group	Donor Cow No.	Plasma Extracted: Time of Collection before (-) or after (+) calving	Plasma Extracted: No. of Calving	Plasma Extracted: Composition: Calcium mg/100 ml.	Plasma Extracted: Composition: Inorganic P. mg/100 ml.	Wt. of Lamb lb	Volume of Plasma Extracted & Infused ml.
1. Control	Q1	-5d		10.40	6.03	36	60
	Q2	-5d	3	12.50	6.12	32	67
	Q5	-10d	6	10.23	4.20	30	80
	Q7	-10d	4	10.94	6.28	30	75
	S2	+4d	3	9.05	4.43	51	100
2. Parturient	Q1	+30min			3.40	36	60
	Q2	+ 1hr	3	11.02	4.00	31	75
	Q5	+10min	6	10.38	5.70	30	85
	Q7	+15min	4	7.92	3.54	34	86
	S2	+ 1hr	3	8.50	3.01	53	69
3. One Day post-partum	S2	+33hr	3	10.40	5.83	40	67
	S2	+33hr	3	10.40	5.83	48	96

TABLE A72

Plasma Calcium Values of Lambs Infused with Ethanol Extracts of Bovine Plasma

mg/100 ml.

Group	Donor Cow No.	Time after Infusion								
		Pre	5 min	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	48 hr
1. Control	Q1	9.81	10.71	12.72	12.19	11.24	10.81			
	Q2	11.15	10.90	10.90	10.90		10.70	10.90	10.80	11.00
	S2	10.45	10.20	10.20	10.15	9.95	9.90	9.95	9.75	10.60
2. Parturient	Q1	11.87	11.02	11.13	9.96	9.65			10.49	
	Q2	9.50	9.70	9.00	8.90	8.90	9.30	9.30	9.00	9.50
	S2	10.00	9.67	9.60	9.55	9.60	10.20	10.20	10.75	11.05

TABLE A73

Plasma Inorganic Phosphate Values of Lambs Infused with Ethanol Extracts of Bovine Plasma

mg/100 ml.

Group	Donor Cow No.	Time after Infusion									
		Pre	5 min	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	36 hr	48 hr
1. Control	Q1	1.61	1.58	2.04	2.75	2.14	1.89		2.27		
	Q2	6.18	4.68	4.51	4.10		3.80	5.08	6.03		4.97
	Q5	6.00						3.39	2.87	4.98	7.56
	Q7	7.91						6.49	8.17	7.91	5.74
	S2	5.27		5.40	5.75	5.62	5.35	5.90	4.95	4.90	5.55
2. Parturient	Q1	4.28	4.35	4.00	4.13		3.33		3.54	3.12	
	Q2	7.38	7.66	7.25	7.46	8.10	8.01	7.15	5.75	5.70	7.16
	Q5	4.55						3.05	4.65	4.05	4.73
	Q7	7.50						6.58	6.36	6.41	5.27
	S2	4.93	3.87	4.52	4.52	5.25	4.70	4.05	4.90	5.13	4.65
3. One Day post-partum	S2	3.78	4.20	3.43	4.55	4.35	4.08	4.10	3.40	3.65	4.10
	S2	4.35	4.10	4.18	4.25	3.80	4.02	5.70	5.95	5.30	5.25

TABLE A74

The effect of administering purified oxytocic principle (450 mu/min) intravenously on the composition of the jugular blood of sheep.

	Sheep No. and Sex	Minutes from Commencement of Experiment								
		Pre-infusion			Period of Infusion			Post-infusion		
		0	10	20	30	40	50	60	70	80
Haemoglobin	1M	0.908	0.954	0.903	0.885	0.862	0.844	0.840	0.871	0.855
	2M	1.005	0.962	0.939	0.853	0.812	0.787	0.803	0.790	0.792
	3F	0.885	0.914	0.831	0.840	0.786	0.753	0.829	0.875	0.860
Uvispek	4F	1.134	1.104	1.080	0.986	0.917	0.925	0.908	0.886	0.878
Reading	5F	1.442	1.282	1.147	1.120	1.132	1.169	1.062	1.103	1.103
	6F	1.308	1.195	1.185	1.241	1.199	1.201	1.147	1.066	1.142
	Mean	1.114	1.069	1.014	0.988	0.951	0.947	0.932	0.932	0.938
Plasma	1M	10.05	9.97	10.03	9.70	9.90	10.03	10.13	10.23	10.20
Calcium	2M	10.59	10.35	10.38	10.35	10.29	10.26	10.26	10.28	10.33
mg/100 ml.	3F	10.32	10.30	10.26	10.22	10.11	9.75	10.259	10.13	10.16
	4F	10.54	10.76	10.51	10.35	10.23	10.39	10.16	10.39	10.35
	5F	9.50	9.36	9.35	9.33	9.30	9.25	9.27	9.18	9.32
	6F	9.67	9.41	9.38	9.46	9.48	9.56	9.42	9.39	9.30
	Mean	10.11	10.03	9.99	9.90	9.89	9.87	9.92	9.93	9.94

TABLE A75

The effect of administering posterior pituitary hormones and oestrogen intravenously on the arterial percentage haematocrit of sheep.

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	21.0	21.0	21.5	22.0	20.0	20.0	19.5	18.5	18.0
	77	18.2	20.0	20.2	18.5	19.5	19.0	19.5	19.5	17.5
	78	23.5	24.0	23.0	23.5	23.5	22.5	24.0	24.0	24.0
	Mean	20.9	21.7	21.6	21.3	21.0	20.5	21.0	20.7	19.8
Purified	31	26.5	25.5	25.5	30.0	27.0	23.5	23.5	24.5	(24.5)
Oxytocic	48	25.0	29.0	27.0	30.0	30.0	27.0	26.5	26.0	26.0
Principle	77	20.5	20.5	21.0	21.5	21.0	21.0	21.0	19.5	19.5
450 mu/min.	78	27.0	24.5	24.5	26.0	27.0	25.5	24.5	23.5	24.5
	Mean	24.8	24.9	24.5	26.9	26.3	24.3	23.9	23.4	23.6
Posterior	31	22.0	22.5	20.0	20.0	20.0	19.5	20.5	20.0	(19.3)
Pituitary	48	27.0	26.0	25.5	23.5	23.0	22.0	22.5	23.0	24.0
≡ 450 mu	77	21.0	22.5	22.0	20.5	21.0	20.0	19.0	18.5	18.0
oxytocin/	78	19.0	19.5	19.5	19.0	19.0	19.5	19.0	18.5	18.0
min		22.3	22.6	21.8	20.8	20.8	20.3	20.3	20.0	19.8
Vasopressin	48	20.0	19.5	18.5	17.0	17.0	16.5	16.5	16.5	16.5
450mu/min	77	17.0	17.5	17.5	15.5	15.5	16.0	15.0	15.0	15.0
	78	20.0	20.3	20.0	18.5	19.0	19.0	19.0	19.0	19.0
	Mean	19.0	19.1	18.7	17.0	17.2	17.2	16.8	16.8	16.8
Stilboestrol	77	19.0	20.3	18.5	18.5	18.0	18.0	17.5	18.5	18.5
20 µg/min	78	25.0	25.5	25.0	24.5	25.0	24.5	24.5	25.0	24.0
	Mean	23.0	22.9	21.8	21.5	21.5	21.3	21.0	21.8	21.3
Purified †	77	17.5	17.0	15.5	18.0	18.0	17.5	17.5	16.0	16.0
Oxytocic	78	23.5	24.5	24.0	26.5	26.5	25.5	24.0	24.0	24.5
Principle										
450 mu/min.	Mean	20.5	20.8	19.8	22.3	22.3	21.5	20.8	20.0	20.3

† 5 mg. stilboestrol given s/c three days previously.
Values in brackets are calculated.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	5	796.91	159.3920	2.02
Within groups	12	947.56	78.9633	
Animals	17	1744.47		
Times	8	62.62	7.8275	9.46***
Groups x Times	40	64.60	1.6150	1.95*
Error	94	77.76	0.8272	
Total	159	1949.45		

TABLE A76

The effect of administering posterior pituitary hormones and oestrogen intravenously on the arterial haemoglobin concentrations of sheep.

(Uvispek readings x 10^3)

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	.771	.812	.815	.812	.766	.800	.721	.698	.698
	77	.900	.970	.991	.925	.963	.926	.940	.937	.833
	78	1.112	1.150	1.103	1.124	1.105	1.076	1.203	1.185	1.135
	Mean	.928	.977	.970	.954	.945	.934	.955	.940	.889
Purified	31	1.036	.963	1.013	1.153	1.026	.942	.880	.941	(.939)
Oxytocic	48	.962	1.135	1.079	1.195	1.159	1.054	.987	.996	.993
Principle	77	1.045	1.024	1.040	1.073	1.039	1.019	.999	.975	.989
450 mu/min.	78	1.201	1.170	1.220	1.252	1.264	1.238	1.172	1.139	1.156
	Mean	1.061	1.073	1.088	1.168	1.122	1.063	1.010	1.013	1.019
Posterior	31	.833	.838	.778	.768	.761	.755	.759	.748	(.712)
Pituitary	48	1.038	1.042	1.001	.904	.887	.876	.877	.879	.921
= 450 mu	77	1.036	1.061	1.028	1.000	.998	.954	.897	.861	.840
oxytocin/min	78	.908	.945	.945	.941	.921	.949	.943	.934	.889
	Mean	.954	.972	.938	.904	.892	.884	.869	.856	.841
Vasopressin	48	.766	.765	.723	.643	.638	.632	.633	.626	.619
450 mu/min.	77	.860	.842	.853	.794	.765	.716	.729	.719	.695
	78	.988	.980	.979	.912	.911	.917	.912	.886	.891
	Mean	.871	.862	.852	.783	.771	.755	.758	.744	.735
Stilboestrol	77	.926	.954	.886	.919	.856	.857	.839	.848	.881
20 µg/min.	78	1.215	1.214	1.197	1.201	1.192	1.170	1.216	1.225	1.158
in oil	Mean	1.070	1.084	1.042	1.060	1.024	1.014	1.028	1.037	1.020
Purified†	77	.835	.806	.785	.876	.860	.840	.799	.758	.761
Oxytocic	78	1.160	1.171	1.153	1.312	1.260	1.209	1.139	1.145	1.153
Principle										
450 mu/min.	Mean	.998	.989	.969	1.094	1.060	1.025	.969	.952	.957

† 5 mg. stilboestrol given s/c three days previously.
Values in brackets are calculated.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	5	1.4621	0.2924	1.44
Within Groups	12	2.4304	0.2025	
Animals	17	3.8925		
Times	8	0.1585	0.0198	13.66***
Groups x Times	40	0.1303	0.0033	2.25**
Error	94	0.1361	0.0015	
Total	159	4.3174		

TABLE A77

The effect of administering posterior pituitary hormones and oestrogen intravenously on the arterial blood calcium concentrations of sheep.

mg/100 ml.

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	8.45	8.59	8.48	8.54	8.57	8.56	8.40	8.55	8.62
	77	8.15	8.22	8.13	8.27	8.06	8.12	7.91	7.80	7.77
	78	7.34	7.14	7.08	6.98	7.02	7.02	7.13	6.94	7.10
	Mean	7.98	7.98	7.90	7.93	7.88	7.90	7.81	7.76	7.83
Purified	31	7.54	7.34	7.32	6.68	6.51	6.37	7.22	7.39	(7.02)
Oxytocic	48	8.22	7.81	7.46	7.19	6.99	6.84	7.10	7.21	7.22
Principle	77	6.98	7.21	6.77	7.13	6.96	7.02	7.08	7.01	7.04
450mu/min	78	6.00	6.18	6.85	6.59	6.22	5.97	6.62	6.48	6.39
	Mean	7.19	7.14	7.10	6.90	6.67	6.55	7.01	7.02	6.91
Posterior	31	7.67	7.86	7.67	7.70	7.38	6.74	7.44	7.61	(7.49)
Pituitary	48	8.12	8.01	8.07	7.93	8.05	7.72	7.60	8.09	7.82
= 450 mu	77	7.81	7.68	7.60	7.90	7.74	7.90	7.55	7.57	7.73
oxytocin/	78	7.59	7.42	7.36	7.65	7.38	7.35	7.29	7.39	7.48
min	Mean	7.80	7.74	7.68	7.80	7.64	7.43	7.47	7.67	7.63
Vasopressin	48	8.69	8.27	8.88	8.84	9.12	9.11	8.52	9.00	8.96
450mu/min	77	8.91	8.15	8.70	8.04	8.60	7.75	8.48	8.48	8.23
	78	5.83	6.27	5.72	5.72	6.17	7.01	6.41	6.45	6.90
	Mean	7.81	7.56	7.77	7.53	7.96	7.96	7.80	7.98	8.03
Stilboestrol	77	6.95	6.70	6.65	6.70	6.98	7.03	7.00	7.11	7.10
20 µg/min	78	(5.84)	5.98	5.85	5.55	5.60	5.45	6.22	6.03	5.70
	Mean	6.40	6.34	6.25	6.13	6.29	6.24	6.61	6.57	6.40
Purified†	77	8.05	8.00	8.37	7.88	8.00	7.78	7.93	8.06	7.75
Oxytocic	78	7.08	6.60	6.70	6.38	6.11	6.03	5.78	5.95	6.32
Principle										
450mu/min	Mean	7.57	7.30	7.54	7.13	7.06	6.91	6.86	7.01	7.04

† 5 mg. stilboestrol given s/c three days previously.
Values in brackets are calculated.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	5	40.3904	8.0781	1.43
Within groups	12	67.7301	5.6441	
Animals	17	108.1205		
Times	8	1.1059	0.1382	3.36**
Groups x Times	40	6.4615	0.1615	3.93***
Error	93	23.8266	0.0411	
Total	158	119.5145		

TABLE A78

The effect of administering posterior pituitary hormones and oestrogen intravenously on the arterial blood magnesium concentrations of sheep.

mg./100 ml.

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	2.96	2.86	2.72	2.70	2.66	2.71	2.74	2.52	2.54
	77	3.39	3.47	3.16	3.15	3.37	3.23	3.31	3.00	2.96
	78	2.50	2.42	2.66	2.37	2.72	2.85	2.93	2.58	2.84
	Mean	2.95	2.92	2.85	2.74	2.92	2.93	2.99	2.70	2.78
Purified	31	3.19	3.14	3.23	3.31	3.11	2.96	2.85	2.98	(3.13)
Oxytocic	48	2.76	2.76	2.85	2.90	2.88	2.83	2.76	2.75	2.78
Principle	77	2.62	2.62	2.60	2.62	2.70	2.72	2.68	2.54	2.62
450 mu/min.	78	2.17	2.00	2.28	2.30	2.45	2.39	2.19	2.37	2.42
	Mean	2.69	2.63	2.74	2.78	2.79	2.73	2.62	2.66	2.74
Posterior	31	2.88	2.86	2.79	2.74	2.71	2.72	2.70	2.74	(2.63)
Pituitary	48	2.50	2.56	2.54	2.38	2.62	2.52	2.47	2.44	2.39
≡ 450 mu	77	3.07	3.03	2.91	2.85	3.11	2.62	2.66	2.56	2.70
oxytocin/min.	78	3.03	3.17	3.01	3.11	3.13	3.18	2.99	3.01	2.93
	Mean	2.87	2.91	2.81	2.77	2.89	2.76	2.71	2.69	2.66
Vasopressin	48	2.62	2.54	2.41	2.52	2.46	2.46	2.40	2.42	2.34
450 mu/min.	77	2.80	2.66	2.55	2.58	2.50	2.46	2.34	2.34	2.24
	78	2.61	2.68	2.66	2.63	2.58	2.58	2.54	2.67	2.60
	Mean	2.68	2.63	2.54	2.58	2.51	2.50	2.43	2.48	2.39
Stilboestrol	77	2.62	2.68	2.74	2.65	2.61	2.49	2.44	2.43	2.46
20 µg/min.	78	2.70	2.88	2.80	2.80	2.64	2.65	2.64	2.64	2.56
in oil	Mean	2.66	2.78	2.77	2.73	2.63	2.57	2.54	2.54	2.51
Purified †	77	2.84	3.08	3.31	3.16	3.02	3.07	3.15	3.08	2.93
Oxytocic	78	2.94	2.75	2.66	2.63	2.76	2.71	2.60	2.55	2.80
Principle	Mean	2.89	2.92	2.99	2.90	2.89	2.89	2.88	2.82	2.86
450 mu/min.										

† 5 mg. stilboestrol given s/c three days previously.
Values in brackets are calculated.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	5	2.3672	0.4734	0.79
Within groups	12	7.2004	0.6000	
Animals	17	9.5676		
Times	8	0.4465	0.0558	3.93***
Groups x Times	40	0.5937	0.0148	1.04
Error	94	1.3433	0.0142	
Total	159	11.9511		

TABLE A79

The effect of administering posterior pituitary hormones and oestrogen intravenously on the arterial blood inorganic phosphate concentrations of sheep.

(mg./100 ml.)

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	4.35	4.49	4.26	4.37	4.20	4.28	4.28	4.26	4.01
	77	4.46	4.37	4.37	4.24	4.15	4.37	3.93	3.78	3.49
	78	4.98	4.67	4.20	4.32	4.02	4.20	4.39	4.78	4.76
	Mean	4.60	4.51	4.28	4.31	4.12	4.28	4.20	4.27	4.09
Purified	31	6.00	5.62	5.44	5.10	5.40	5.40	5.06	4.96	(5.12)
Oxytocic	48	4.98	4.90	4.58	4.60	4.88	4.85	4.81	4.81	4.52
Principle	77	4.63	4.46	4.31	4.03	3.73	3.51	3.53	3.81	3.97
450 mu/min.	78	4.59	4.74	4.76	4.00	3.69	3.63	3.37	3.28	3.57
	Mean	5.05	4.93	4.77	4.43	4.43	4.35	4.19	4.22	4.30
Posterior	31	5.30	5.04	4.86	4.82	5.14	4.72	4.24	4.18	4.37
Pituitary	48	5.21	5.25	5.19	5.33	5.25	5.23	5.25	5.23	5.25
= 450 mu	77	4.75	4.70	4.63	4.78	4.48	4.26	3.87	3.77	3.60
oxytocin/min.	78	6.08	5.70	5.44	5.38	5.10	5.02	4.74	4.36	4.24
	Mean	5.34	5.17	5.03	5.08	4.99	4.81	4.53	4.39	4.37
Vasopressin	48	4.65	4.71	4.81	5.06	5.12	5.16	5.00	4.96	4.92
450 mu/min.	77	5.95	5.95	5.83	6.20	6.10	5.88	5.85	5.78	5.63
	78	4.17	4.00	4.28	4.29	4.35	4.00	3.85	3.76	3.96
		4.92	4.89	4.97	5.18	5.19	5.01	4.90	4.83	4.84
Stilboestrol	77	2.76	2.55	2.61	2.99	2.93	2.95	3.44	2.91	2.86
20 µg/min.	78	4.70	4.18	4.30	4.45	4.42	4.60	4.63	4.55	4.88
in oil	Mean	3.73	3.37	3.46	3.72	3.68	3.78	4.04	3.73	3.87
Purified †	77	5.13	5.04	5.15	4.70	4.00	3.54	3.24	3.07	3.17
Oxytocic	78	4.38	3.93	4.05	3.60	3.08	2.88	2.50	2.68	2.90
Principle	Mean	4.76	4.49	4.60	4.15	3.54	3.21	2.87	2.88	3.04
450 mu/min.										

† 5 mg. stilboestrol given s/c three days previously.
Value in brackets is calculated.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	5	33.4652	6.6930	1.70
Within groups	12	47.1799	3.9317	
Animals	17	80.6451		
Times	8	8.3239	1.0405	16.23***
Groups x Times	40	10.2964	0.2574	4.02***
Error	94	6.0275	0.0641	
Total	159	105.2929		

TABLE A80

The effect of administering posterior pituitary hormones intravenously on the percentage haemoglobin differences (hepatic veno-arterial) of sheep.

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	0.1	3.4	0.7	2.1	2.4	0.5	1.7	1.3	1.3
	77	6.9	7.1	8.7	6.9	8.1	5.9	5.5	4.8	3.5
	78	0.5	0.0	2.2	-1.0	0.0	-0.4	2.8	3.7	0.4
	Mean		3.29			2.72			2.78	
Purified	31	3.4	-0.5	2.1	1.6	1.2	2.7	-1.0	-0.6	
Oxytocic	48	0.0	-1.3	-0.6	-0.1	3.2	0.8	1.3	1.8	0.3
Principle	77	1.9	4.0	1.4	3.2	1.5	0.3	1.2	-0.4	0.8
450 mu/min.	Mean		1.16			1.60			0.43	
Posterior	31	1.8	0.8	0.0	0.9	0.8	0.7	0.3	0.7	
Pituitary	48	1.5	2.2	2.8	0.7	1.0	1.0	0.0	-1.3	-1.1
≡ 450 mu	78	0.3	0.2	0.1	0.2	-0.6	-1.7	-0.1	0.6	-0.6
oxytocin/min.	Mean		1.08			0.33			-0.17	
Vasopressin	48	1.5	4.9	3.9	0.0	0.0	-0.6	-0.5	0.6	0.8
450 mu/min.	77	2.9	1.4	1.8	-0.5	1.3	-1.8	3.0	2.8	1.0
	78	3.0	2.6	2.7	1.2	-1.1	-0.9	0.3	0.5	0.0
	Mean		2.74			-0.27			0.94	
Purified†	77	5.8	3.9	4.7	0.2	2.9	1.8	3.1	5.9	3.6
Oxytocic	78	3.0	3.0	1.5	2.3	2.8	1.1	0.7	2.0	2.3
Principle										
450 mu/min.	Mean		3.65			1.93			2.93	

† 5 mg. stilboestrol given s/c three days previously.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	4	97.04	24.26	1.20
Within groups	9	181.61	20.18	
Animals	13	278.65		
Times	2	4.51	2.26	1.44
Groups x Times	8	61.26	7.66	4.88***
Error	100	157.11	1.57	
Total	123	501.53		

TABLE A81

The effect of administering posterior pituitary hormones intravenously on the calcium corrected hepatic veno-arterial differences of sheep.

mg./100 ml.

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	0.15	0.19	0.10	0.06	0.07	-0.12	0.21	0.00	0.25
	77	0.47	0.49	0.78	0.61	0.51	0.32	0.32	0.36	0.32
	78	-0.35	0.05	-0.03	-0.22	-0.01	-0.06	0.14	0.06	-0.24
	Mean		0.21			0.13			0.16	
Purified	31	0.15	0.23	0.21	0.59	0.64	0.86	0.12	-0.03	
Oxytocic	48	0.03	-0.12	0.08	0.05	0.38	0.36	-0.13	0.13	0.09
Principle	77	-0.01	0.40	0.14	0.02	0.12	0.23	-0.02	-0.12	-0.29
450 mu/min.	Mean		0.12			0.36			-0.03	
Posterior	31	0.49	0.19	0.08	0.30	0.02	0.34	-0.03	-0.01	
Pituitary	48	-0.05	-0.04	0.14	0.15	-0.16	0.13	0.21	-0.11	-0.13
≡ 450 mu	78	-0.19	-0.15	0.03	-0.28	-0.18	-0.17	-0.03	-0.10	-0.08
oxytocin/min.	Mean		0.06			0.02			-0.03	
Vasopressin	48	0.10	0.82	0.55	0.19	-0.10	-0.27	0.59	0.16	0.39
450 mu/min.	77	-0.29	-0.29	-0.20	0.44	0.04	0.14	0.07	-0.15	-0.07
	78	0.05	-0.14	0.19	0.38	-0.33	-0.06	-0.29	-0.09	-0.20
	Mean		0.09			0.05			0.05	
Purified †	77	0.37	0.34	0.12	0.11	0.13	0.09	0.06	0.31	-0.14
Oxytocic	78	-0.23	0.46	-0.17	0.37	0.75	-0.20	0.17	-0.04	0.00
Principle										
450 mu/min.	Mean		0.15			0.21			0.06	

† 5 mg. stilboestrol given s/c three days previously.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	4	0.4562	0.1141	0.37
Within groups	9	2.7543	0.3060	
Animals	13	3.2105		
Times	2	0.2537	0.1269	3.03 ϕ
Groups x Times	8	0.5536	0.0692	1.65 ϕ
Error	100	4.4195	0.0442	
Total	123	8.4373		

ϕ $P \simeq 0.10$

TABLE A82

The effect of administering posterior pituitary hormones intravenously on the magnesium corrected hepatic veno-arterial differences of sheep.

mg./100 ml. blood

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	-0.14	-0.07	-0.03	0.07	-0.01	-0.02	-0.16	0.13	0.03
	77	0.12	-0.01	0.37	0.25	-0.08	0.30	-0.10	0.16	0.19
	78	0.31	0.57	-0.19	0.03	0.08	-0.12	-0.10	-0.09	-0.26
	Mean		0.10			0.06			-0.02	
Purified	31	0.09	0.00	-0.05	0.07	0.02	0.07	-0.01	-0.04	
Oxytocic	48	0.10	0.14	0.03	-0.04	0.04	-0.04	-0.06	0.03	-0.04
Principle	77	0.07	0.05	0.11	0.19	0.02	-0.08	-0.06	-0.08	-0.07
450 mu/min.	Mean		0.06			0.03			-0.04	
Posterior	31	0.06	0.05	-0.05	0.02	0.01	0.00	0.01	-0.01	
Pituitary	48	0.05	-0.05	0.05	0.14	-0.08	-0.18	0.01	0.03	0.04
≡ 450 mu	78	0.05	-0.07	-0.08	0.01	-0.08	-0.12	0.06	0.00	-0.10
oxytocin/min.	Mean		0.00			-0.03			0.00	
Vasopressin	48	0.00	0.12	0.10	-0.05	0.01	-0.09	-0.09	-0.11	-0.10
450 mu/min.	77	-0.02	0.02	0.08	-0.01	-0.01	-0.06	-0.03	0.03	0.14
	78	0.11	-0.03	-0.03	0.09	-0.03	0.01	0.00	-0.16	-0.01
	Mean		0.04			-0.02			-0.04	
Purified †	77	0.39	0.09	0.09	-0.07	0.21	-0.14	0.09	0.07	0.10
Oxytocic	78	0.05	0.12	0.09	0.08	0.08	-0.07	-0.05	-0.10	0.00
Principle										
450 mu/min.	Mean		0.09			0.01			0.01	

† 5 mg. stilboestrol given s/c three days previously.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	4	0.0803	0.0201	1.18
Within groups	9	0.1544	0.0171	
Animals	13	0.2347		
Times	2	0.1400	0.0700	5.56**
Groups x Times	8	0.0706	0.0088	0.70
Error	100	1.2590	0.0126	
Total	123	1.7043		

TABLE A83

The effect of administering posterior pituitary hormones intravenously on the inorganic phosphate corrected hepatic veno-arterial differences of sheep.

mg./100 ml. blood

Treatment	Sheep No.	Minutes								
		Pre-injection			Injection Period			Post-injection		
		0	10	20	30	40	50	60	70	80
Control	48	-0.02	0.27	-0.08	0.30	0.10	0.08	0.19	0.24	0.02
	77	0.63	0.64	0.72	0.51	0.54	0.10	0.24	0.18	0.23
	78	-0.26	-0.06	0.17	-0.10	0.22	0.17	0.28	-0.04	0.24
	Mean		0.22			0.21			0.18	
Purified	31	0.20	-0.08	0.16	0.16	0.17	0.31	0.11	-0.07	
Oxytocic	48	-0.04	-0.40	-0.03	0.00	-0.06	0.23	-0.02	0.09	0.18
Principle	77	0.29	0.43	0.28	0.26	0.21	0.08	0.24	0.14	0.24
450 mu/min.	Mean		0.09			0.15			0.10	
Posterior	31	0.07	0.08	0.08	-0.14	-0.02	0.19	0.03	0.10	
Pituitary	48	-0.03	0.14	0.21	-0.04	0.05	0.05	0.02	0.03	0.00
= 450 mu	78	-0.37	-0.11	0.09	-0.11	-0.01	-0.07	0.10	-0.07	-0.10
oxytocin/min.	Mean		0.02			-0.01			0.01	
Vasopressin	48	0.13	0.34	0.36	0.02	0.03	-0.03	0.14	0.09	0.08
450 mu/min.	77	0.58	0.47	0.48	0.20	0.11	-0.11	0.07	0.24	0.03
	78	0.24	0.18	0.00	0.28	-0.14	0.16	-0.27	0.22	-0.44
	Mean		0.31			0.06			0.02	
Purified †	77	0.53	0.38	0.18	-0.12	0.04	-0.13	-0.15	0.34	0.09
Oxytocic	78	0.41	0.53	0.19	0.11	0.16	-0.20	0.07	0.21	0.02
Principle										
450 mu/min.	Mean		0.37			-0.02			0.10	

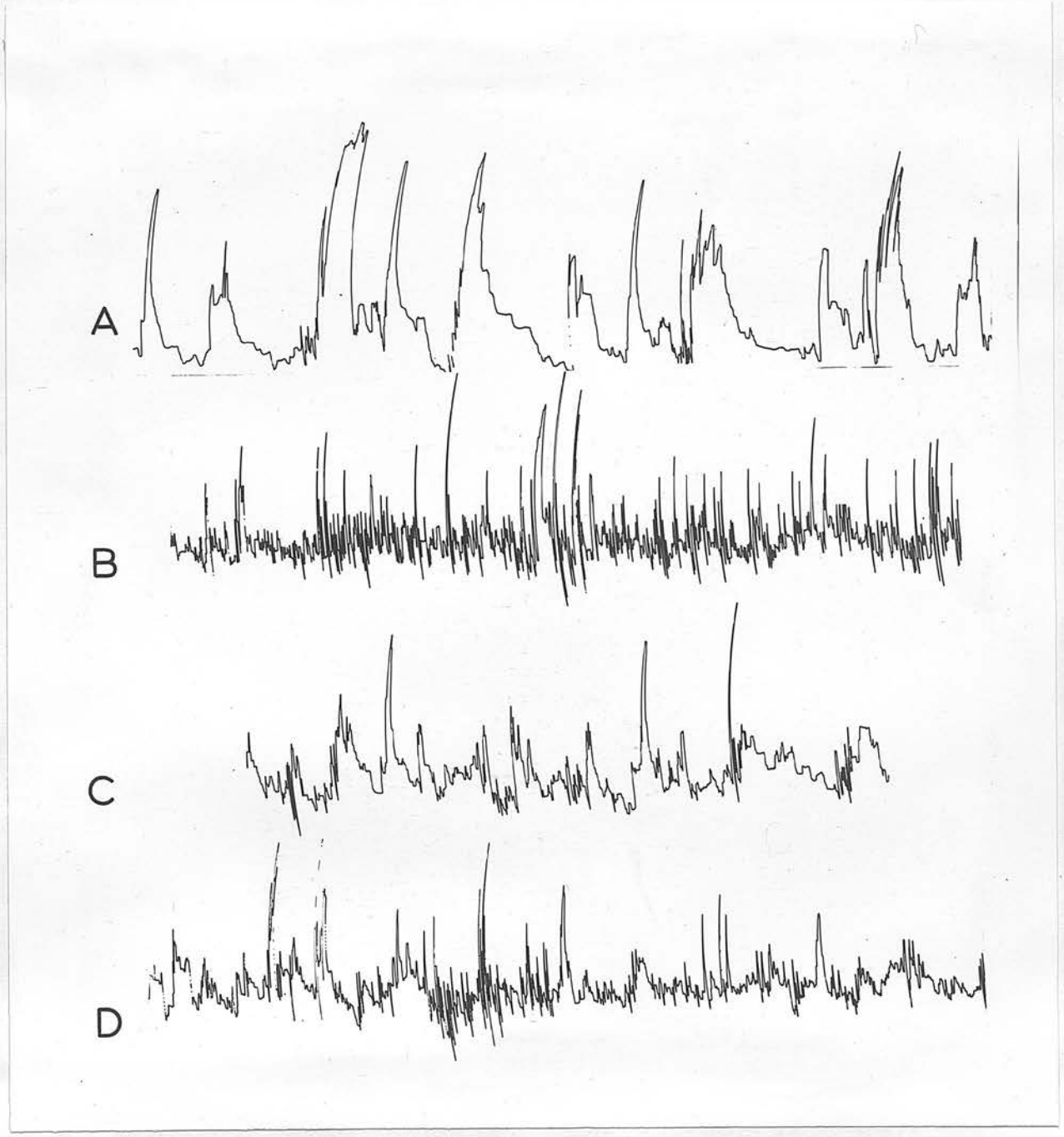
† 5 mg. stilboestrol given s/c three days previously.

Analysis of Variance

Source of Variation	d.f.	Sums of Squares	Mean Square	F
Groups	4	0.5411	0.1353	1.01
Within groups	9	1.2026	0.1336	
Animals	13	1.7437		
Times	2	0.3111	0.1556	6.03**
Groups x Times	8	0.6577	0.0822	3.19**
Error	100	2.5839	0.0258	
Total	123	5.2964		

APPENDIX II

FIGURE A1 Rumen movements recorded from the left paralumbar fossa of cow F4, injected with hyoscine hydrobromide $^{s}/_{c}$.



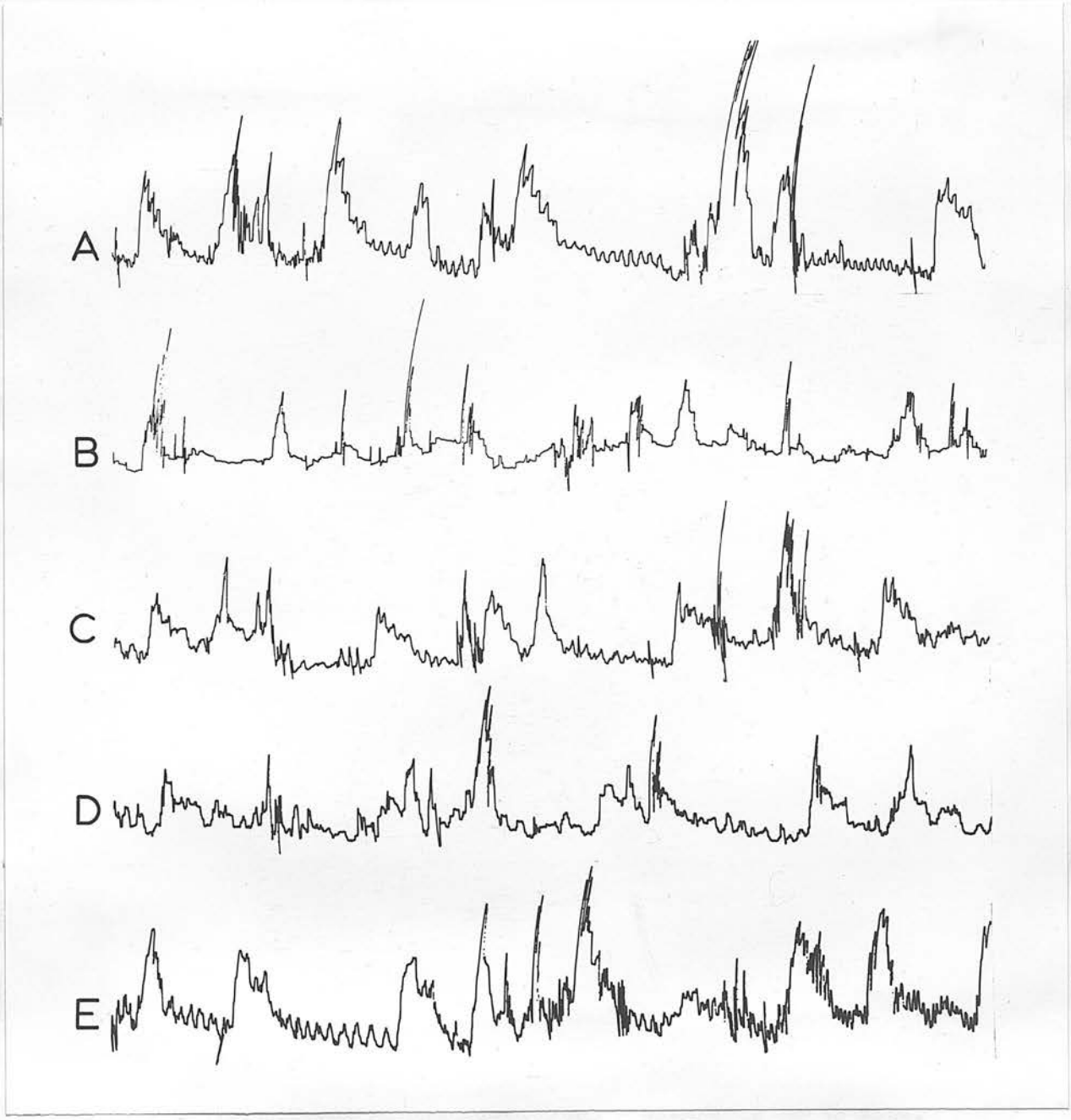
A - Twenty-four hours before initial injection. Movements 20/10 mins. Sounds score 2.0.

B - Four hours after initial injection. Movements 2/10 mins? Sounds score 0.5.

C - Twelve hours after initial injection. Movements 11/10 mins. Sounds score 0.

D - Eighteen hours after initial injection and six hours after maintenance dose. Movements 10/10 mins? Sounds score 0.5.

FIGURE A2 Rumen movements recorded from the left paralumbar fossa of cow D19, injected with hyoscine hydrobromide $^{s}/_{c}$.



A - Immediately before initial injection. Movements 16/10 mins. Sounds score 3.0.

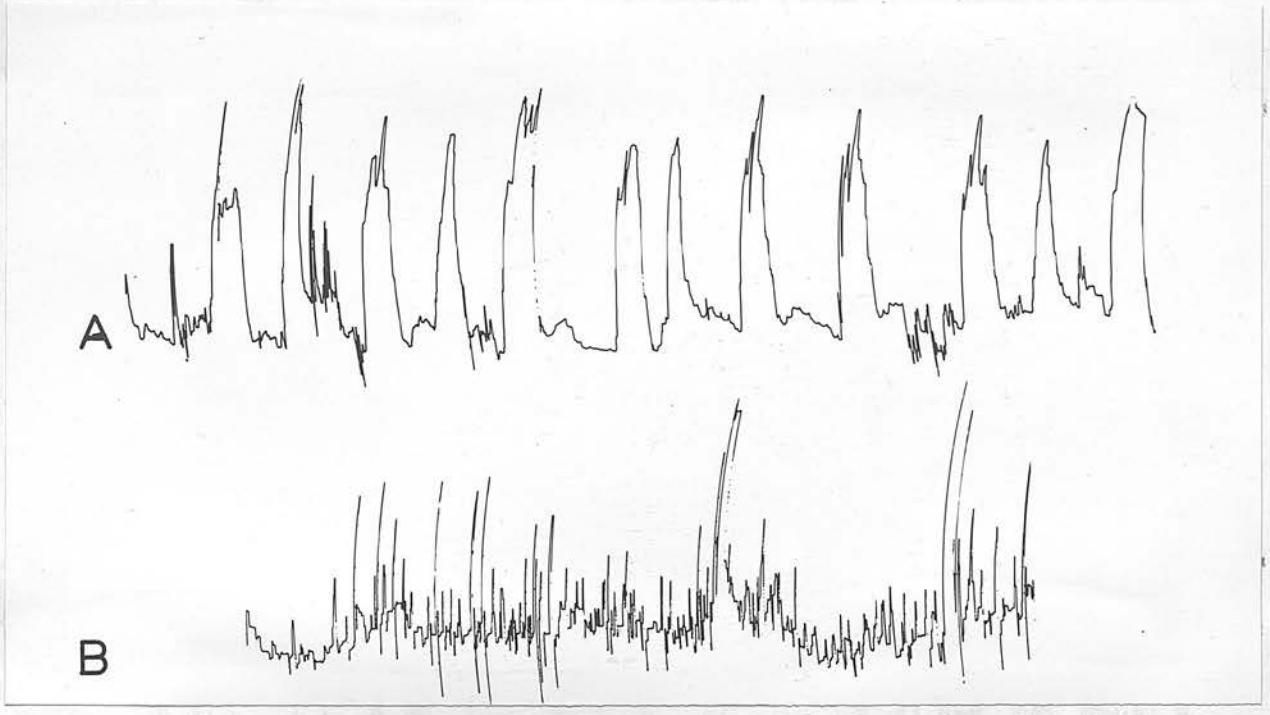
B - Eight hours after initial injection. Movements 6/10 mins. Sounds score 0.

C - Twelve hours after initial injection. Movements 16/10 mins. Sounds score 1.0.

D - Eighteen hours after initial injection, six hours after maintenance dose. Movements 13/10 mins. Sounds score 0.5.

E - Twenty-four hours after initial injection and twelve hours after maintenance dose. Movements 18/10 mins. Sounds score 2.0.

FIGURE A3 Rumen movements recorded from the left paralumbar fossa of cow E14, injected with hyoscine hydrobromide s/c.

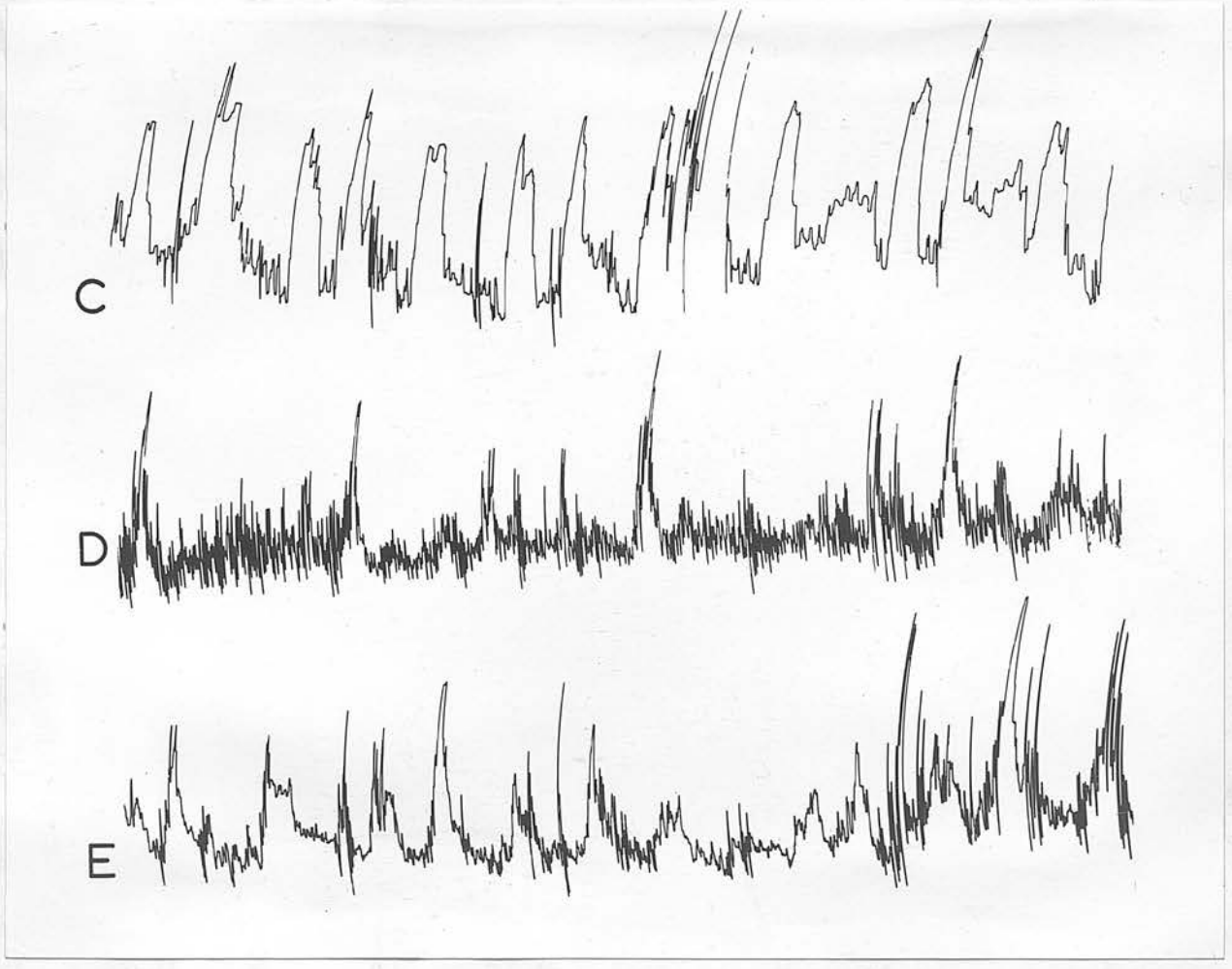


A - Immediately before injection. Movements 22/10 mins.
Sounds score 2.0.

B - Four hours after initial injection. Movements 3/10 mins.
Sounds score 0.

(Continued Overleaf)

FIGURE A3 (cont'd)

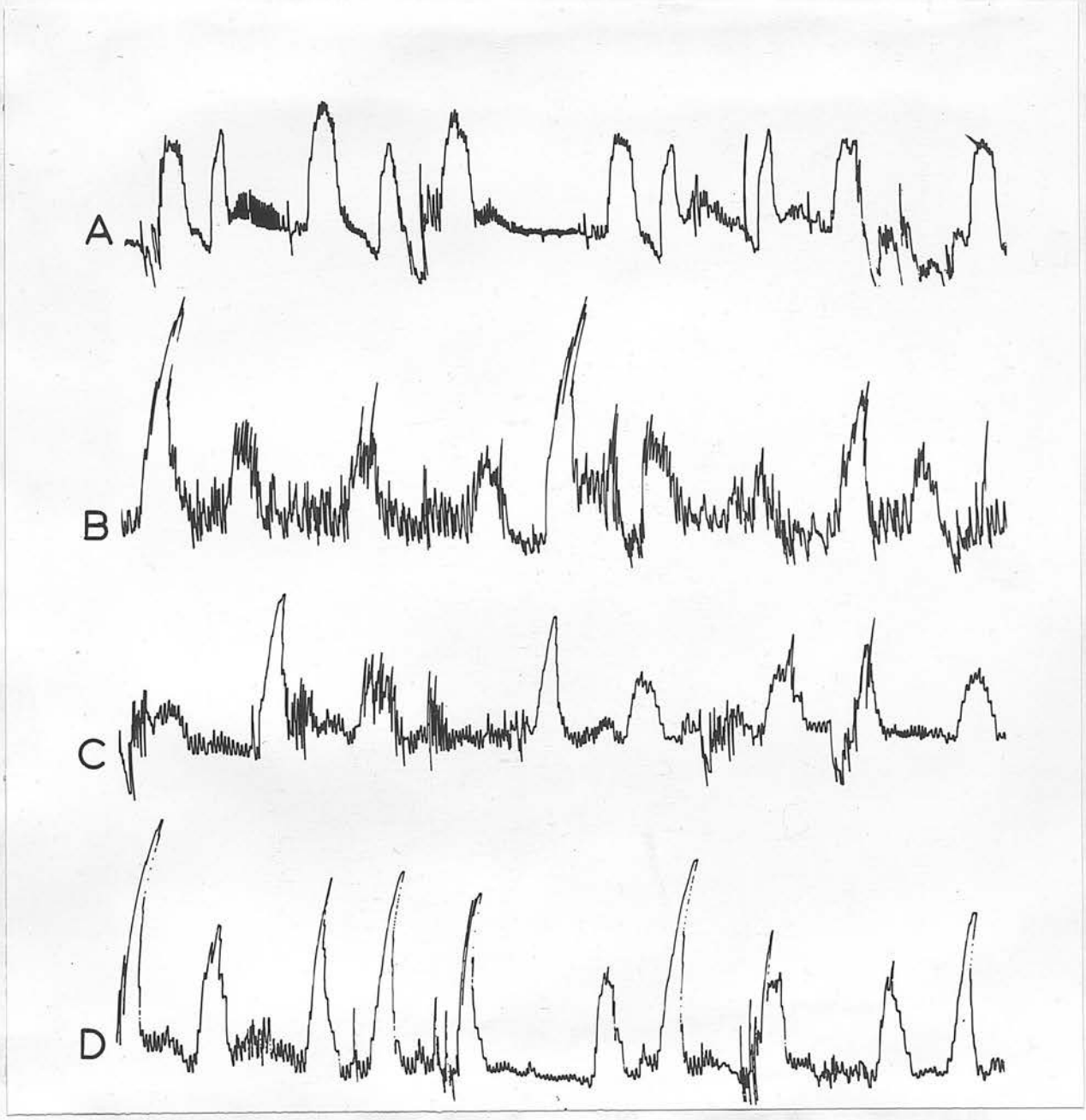


C - Eight hours after initial injection. Movements 22/10 mins. Sounds score 0.5.

D - Twelve hours after initial injection and four hours after second injection. Movements 6/10 mins. Sounds score 1.0.

E - Eighteen hours after initial injection and six hours after third injection. Movements 20/10 mins. Sounds score 0.5.

FIGURE A4 Rumen movements recorded from the left paralumbar fossa of cow B51, injected with hyoscine hydrobromide $^{s}/_{c}$.



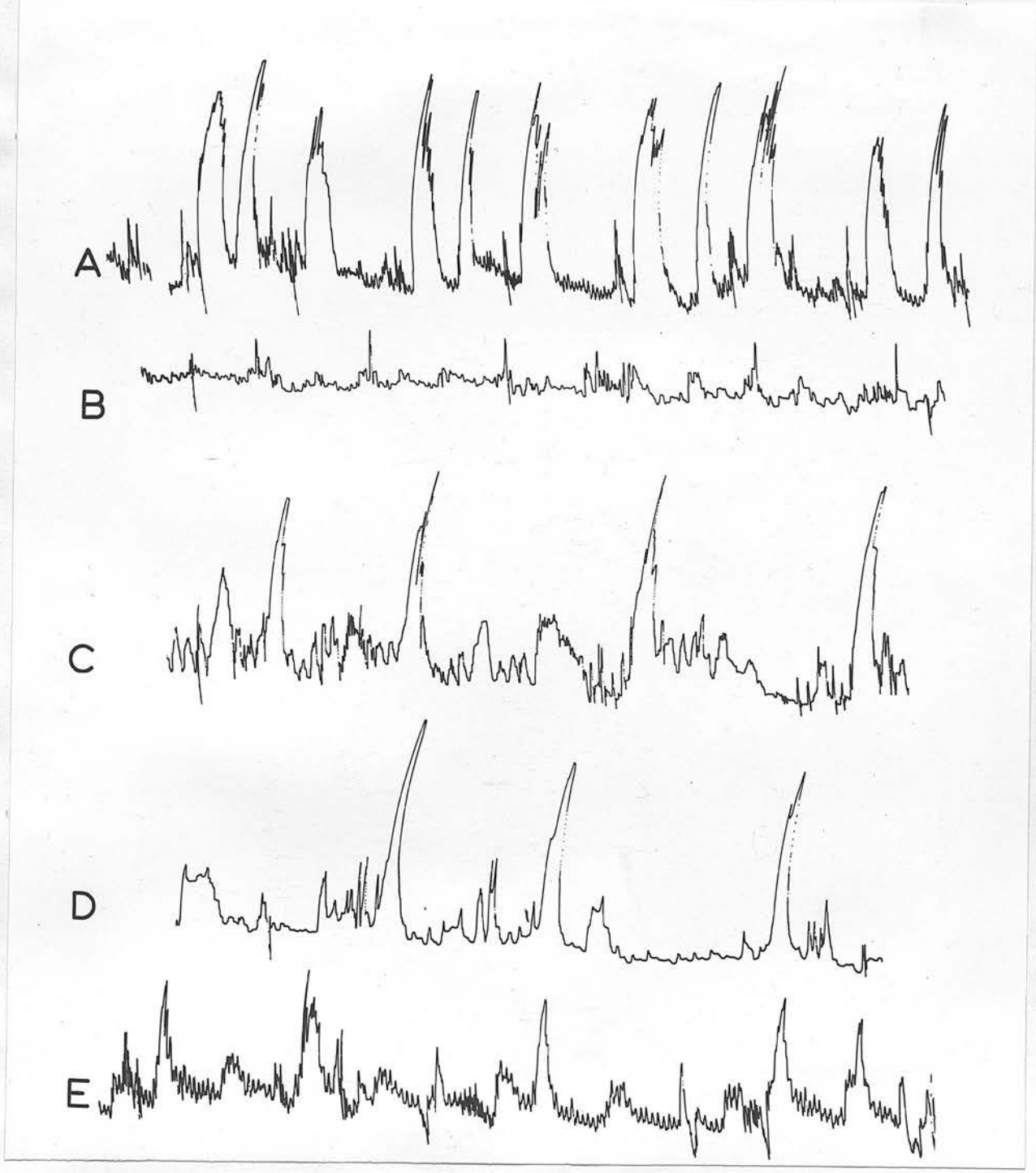
A - Immediately before injection. Movements 17/10 mins. Sounds score 2.5.

B - Ten hours after initial injection. Movements 17/10 mins. Sounds score 0.5.

C - Twenty-four hours after initial injection. Movements 15/10 mins. Sounds score 1.5.

D - Thirty hours after initial injection. Movements 17/10 mins. Sounds score 0.5.

FIGURE A5 Rumen movements recorded from the left paralumbar fossa of cow P11, injected with hyoscine hydrobromide $^{s}/_{c}$.



A - Immediately before injection. Movements 20/10 mins. Sounds score 3.0.

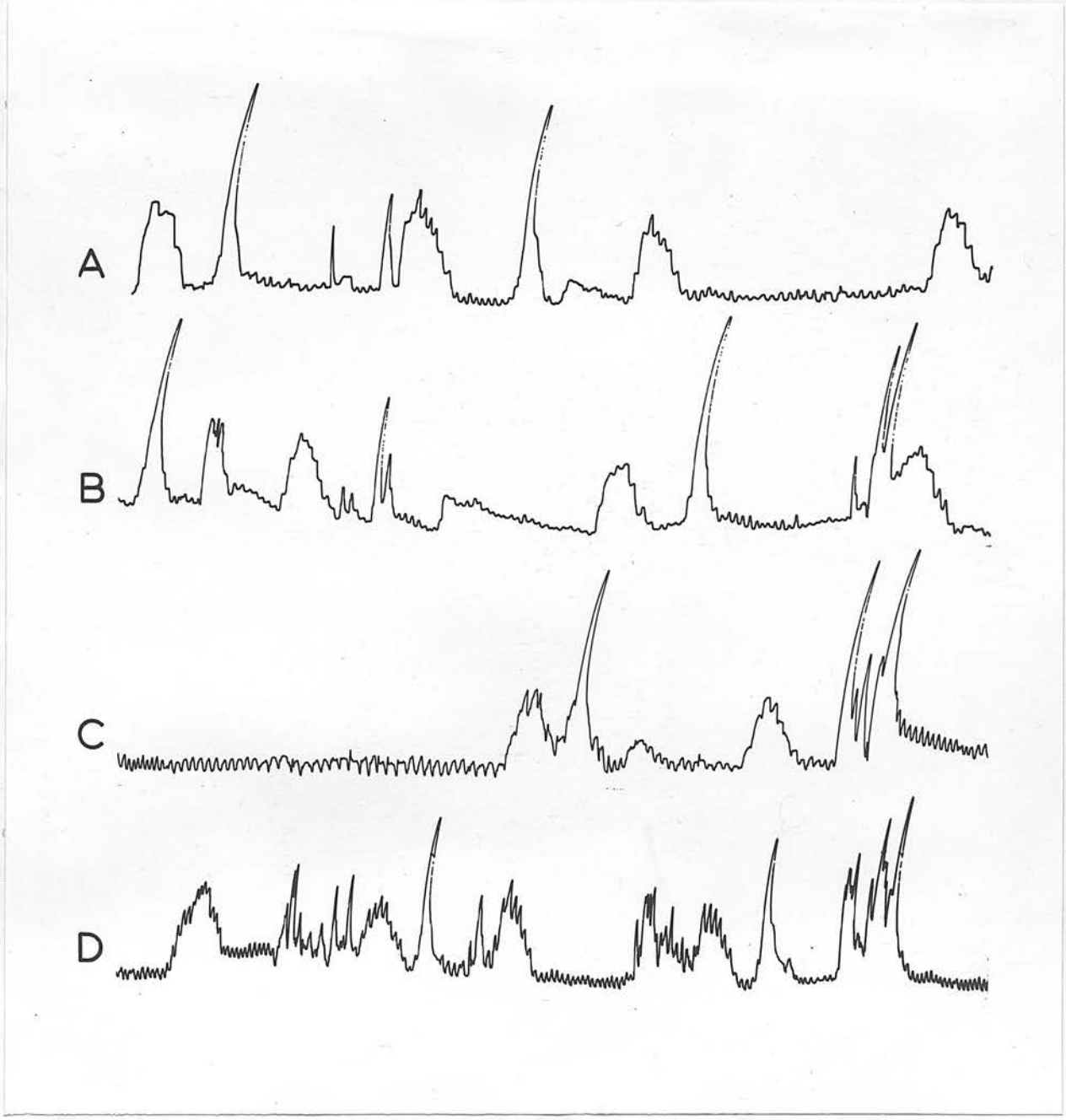
B - Twelve hours after injection. Movements 0. Sounds score 0.

C - Twenty hours after hyoscine injection and one hour after calcium therapy. Movements 12/10 mins. Sounds score 2.5.

D - Twenty-four hours after hyoscine injection and five hours after calcium therapy. Movements 10/10 mins. Sounds score 3.0.

E - Thirty-six hours after hyoscine injection and seventeen hours after calcium therapy. Movements 22/10 mins. Sounds score 2.5.

FIGURE A6 Rumen movements recorded from the left paralumbar fossa of a heifer injected with posterior pituitary extract equal to 0.15 oxytocic units per kilogram $^{s}/_{c}$.



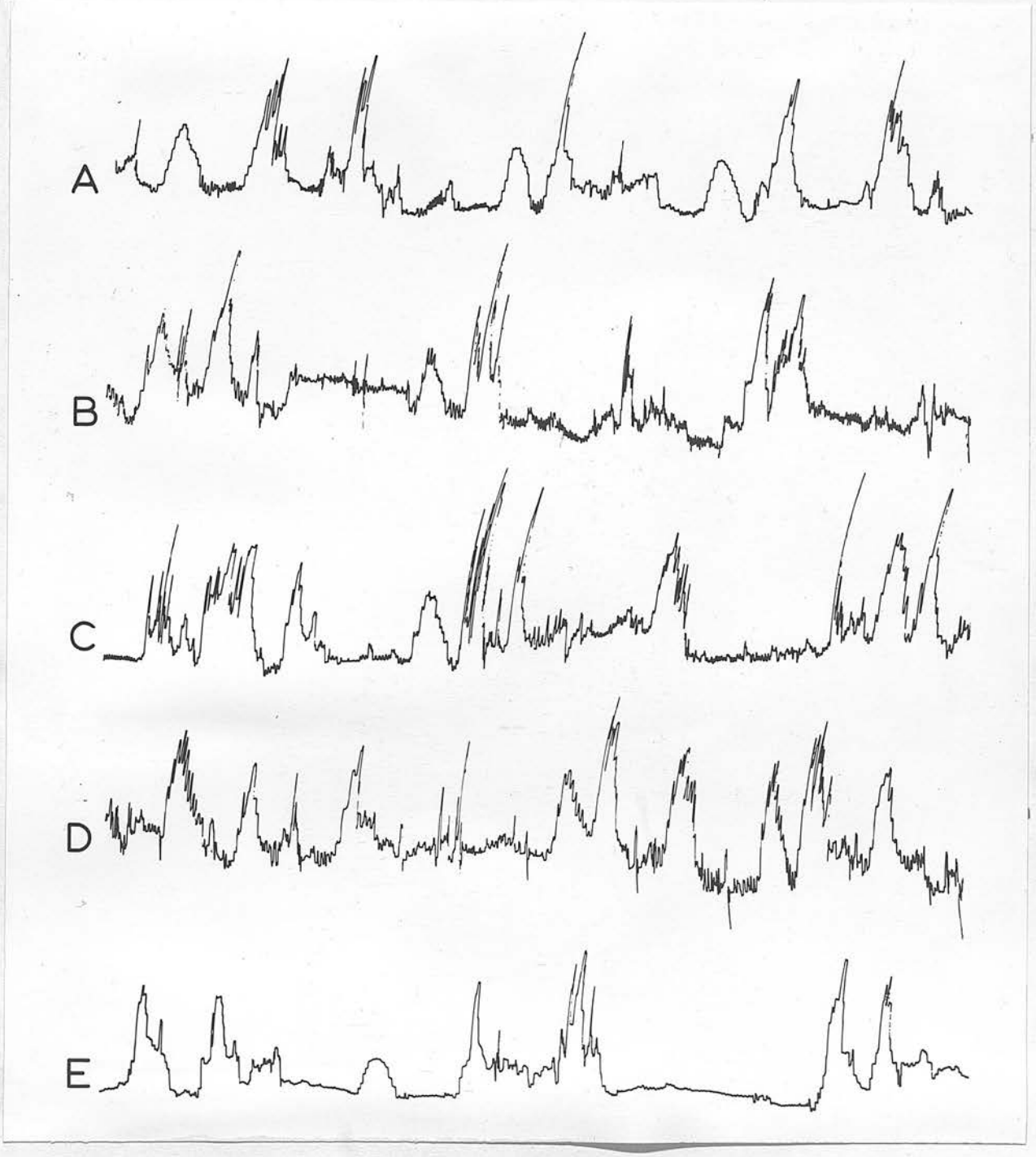
A - Before injection. Movements 15/10 mins. Sounds score 2.0.

B - Three to seven minutes after injection. Movements 13/10 mins. Sounds score 2.0.

C - Thirty minutes after injection. Movements 12/10 mins. Sounds score 2.0.

D - Two hours after injection. Movements 16/10 mins. Sounds score 2.0.

FIGURE A7 Rumen movements recorded from the left paralumbar fossa of a cow injected with posterior pituitary extract equal to 0.25 oxytocic units per kilogram $^{s}/_{c}$.



A - Before injection. Movements 18/12 mins.

B - Two to four minutes after injection. Movements 15/10 mins.

C - Thirty minutes after injection. Movements 18/10 mins.

D - One hour after injection. Movements 22/10 mins.

E - Two hours after injection. Movements 17/10 mins.